EAACI
EUROPEAN ACADEMY OF ALLERGY AND CLINICAL IMMUNOLOGY

The European Academy Allergy Monographs

HYMENOPTERA VENOM ALLERGY

Allergy School Proceedings
Ancona, Italy, 2009

European Academy of Allergy and Clinical Immunology (EAACI)

Scientific Committee:
M. Beatrice Biló, Chairperson, EAACI Interest Group - Insect Venom Hypersensitivity
Franziska Ruëff, Secretary, EAACI Interest Group - Insect Venom Hypersensitivity

Editor:
Markus Ollert

EUROPEAN ACADEMY OF ALLERGY
AND CLINICAL IMMUNOLOGY

The European Academy
Allergy Monographs

HYMENOPTERA VENOM ALLERGY

Allergy School Proceedings
Ancona, Italy, 2009

European Academy of Allergy and Clinical Immunology (EAACI)

Scientific Committee:
M. Beatrice Biló, Chairperson, EAACI Interest Group - Insect Venom Hypersensitivity
Franziska Ruëff, Secretary, EAACI Interest Group - Insect Venom Hypersensitivity

Editor:
Markus Ollert

HYMENOPTERA VENOM ALLERGY

Allergy School Proceedings **June 2010**

CONTENTS

PREFACE

Insect sting allergy and venom immunotherapy represent an excellent model for the study of anaphylaxis and immune tolerance. Many aspects of immediate type allergy can be studied. The availability of high quality venom extracts for use in the diagnosis and treatment of insect venom allergy has dramatically improved the prognosis and the health-related quality of life of venom-allergic patients. However, many patients are still not adequately treated. The lack of compensation of treatment costs by health insurance companies may play a role in some European countries, but often the inadequate treatment is due to ignorance. We hope that this booklet may at least help to improve medical knowledge on diagnosis and treatment of Hymenoptera venom allergy.

In recent years, new insights have emerged into both the immunological mechanisms of venom immunotherapy and the natural history and risk factors of severe systemic reactions in untreated as well as treated patients. Even if there are many open questions today there is enough evidence-based knowledge to give the working clinical allergist an arsenal of instruments to help in deciding who and how best to treat with venom immunotherapy.

This progress resulted in the first Allergy School on Insect Sting Allergy in Italy, whose purpose was primarily to educate and promote relations among young European physicians who were interested in the different aspects of venom allergy. Indeed, the course not only covered a series of basic and advanced level lectures, but also interactive practical demonstrations as well as the presentation and discussion of difficult case reports. In addition, several studies were presented in a poster session. The Allergy School in Ancona was attended by a large number of Italian and International specialists alike, many of them being members of the EAACI Interest Group on Insect Venom Hypersensitivity and Allergy Diagnostics.

The following congress proceedings bring all the new insight of this Allergy School to the fore, the most controversial areas of the field are discussed, and answers to many important questions are provided for those who are active in the field of insect venom allergy.

We are sure that Hymenoptera venom allergic patients will benefit from a further increase of knowledge in this field and hope that this booklet may contribute to improved care.

M.Beatrice Bilò
Chairperson of the EAACI Interest Group
on Insect Venom Hypersensitivity

Franziska Ruëff
Secretary of the EAACI Interest Group
on Insect Venom Hypersensitivity

ACKNOWLEDGEMENTS

We sincerely hope the Allergy School has proved useful to and stimulating for physicians who are considering specialising in the field of venom allergy, and that it has provided the necessary insight to those already involved in this field for the continuation of their daily occupation and research.
We wish to extend our thanks to the Insect Venom Hypersensitivity Interest Group of the EAACI and all the speakers for their invaluable contribution and the high standard of their work. We thank the EAACI Allergy Diagnostics Interest Group for its help and cooperation and the AAITO (The Italian Association of Allergists and Immunologists) for their patronage. Thanks also to the European Academy, especially Prof. Luis Delgado and the EAACI Headquarters. Finally, we are indebted to ALK for the unrestricted educational grant that supported these monographs and to Prof. Markus Ollert for his role as editor.

M.Beatrice Bilò and Floriano Bonifazi
Local Organisers
University Hospital - Ospedali Riuniti di Ancona, Italy

FOREWORD

For more than a decade now, the European Academy of Allergy and Clinical Immunology, has organised several Allergy Schools each year. These are educational events for European clinicians and scientists working in the field of allergy, and provide continuous professional training and development in general or specific areas of allergy & immunology for junior, in-training, and full EAACI members. As part of this framework, The EAACI & GA²LEN Allergy School on Hymenoptera Venom Allergy took place in Ancona, Italy, from the 3rd-6th September 2009, organised by the EAACI Interest Group on Hymenoptera Venom Hypersensitivity, with the collaboration of the EAACI Allergy Diagnostics Interest Group and support of the Italian Association of Allergists and Immunologists (AAITO).

The motto proposed by the scientific program coordinators for this EAACI Allergy School was "If you want to become a good allergist, start by studying Hymenoptera venom allergy and venom immunotherapy!". In fact, the program, which focused on Hymenoptera venom allergy, addressed several aspects that are today key issues for the practicing allergist, including understanding of the epidemiology, the interaction of concomitant factors in the clinical expression of allergy (e.g. environment, genetics, co-morbidities and their treatment), decision making and use of the appropriate diagnostic tools (from skin tests, cellular and molecular approaches to challenge tests), patient selection for immunotherapy/allergy vaccination, the treatment of anaphylaxis and, last but not least, quality-of-life issues for the appraisal of our practice outcomes. Moreover, the Ancona EAACI & GA²LEN Allergy School was able to unite 15 different nationalities, bringing together key European experts in the field of Hymenoptera allergy, EAACI Junior members, scientists, and practicing allergists, in a unique informal and interactive educational environment.

Profiting from the unique educational material organised for the school by the faculty and participants, and a generous unrestricted grant from ALK, we put forward this first initiative of "The European Academy Allergy Monographs". So, dear reader, enjoy this update and state-of-the art booklet on Hymenoptera Venom Allergy. Although you cannot experience the hands-on practical side of our Allergy School (and, alas, the cultural and social joys of Ancona!), I am sure these proceedings will provide you with valuable information for your allergy practice and knowledge of the challenging field of venom allergy and anaphylaxis. I also hope to see you at our future EAACI Allergy Schools, so that you may profit from such an excellent educational experience as the Ancona School!

Luis Delgado
EAACI Vice-President, Education & Specialty

INTRODUCTION
History of insect sting allergy

Ulrich R. Müller

Allergiestation Medizinische, Klinik Ziegler Spital, Bern, Switzerland

The first species of Hymenoptera appeared around 200 million ago and were saprophages that deposed their eggs in decaying organic material that served as food for the larvae (Fig. 1). During the 50 million years that followed, Hymenoptera evolved into carnivores and their ovipositor was transformed into a stinging apparatus with venom glands; these were the first wasps. Descendants of these carnivorous wasps began feeding on pollen and nectar, giving rise to the honeybees.

The earliest honeybee specimen was found imbedded in amber and dates from about 80 million years ago (1). The appearance of *homo sapiens* in the last 100-200,000 years (2) meant that conflicts between humans and Hymenoptera for honey and other food are likely to have been commonplace for the last 100,000 years. The first documented evidence of such a conflict, which probably resulted in *homo sapiens* receiving painful stings, is found in Spanish cave paintings that date from around 8000 BC. These show a honey collector running away from angry honeybees (3).

The first, albeit not very convincing, report on a fatal sting reaction dates back to the year 2641 BC when the Egyptian pharaoh Menes was stung by a Kheb while landing on an island in the Atlantic ocean (probably England) and died shortly afterwards. Although the hieroglyph for Kheb shows some similarity with a wasp or a hornet, its interpretation is heavily debated. According to some egyptiologists the tragic event was caused by a hippopotamus and more likely occurred on an island in the Nile river than in the Atlantic ocean (4).

Severe, sometimes fatal sting reactions have been reported in detail since the 17th century. The first to describe rather unusual symptoms, was reported in 1699 by the Bavarian monk Ulrich Staudigl (5), this was followed by a more detailed description of a fatal reaction, by the French physician Debrest in 1765 (6). These initial reports were followed by reports from all over Europe and US. As early as the beginning of the 20th century, such reactions were already recognised as the consequence of an abnormal immune response, and were defined as anaphylaxis by Portier and Richet in 1902 (7) and allergy by von Pirquet 1906 (8). In 1911 Freeman and Noon (9) reported successful vaccination of individuals with pollen allergy using pollen vaccine, and in 1925 the South African physician Braun was the first to successfully desensitise a patient who was allergic to bee stings with an extract from bee venom sacs, to which the patient had positive skin tests (10). In 1930 Robert Benson described the case of a beekeeper who suffered hay fever symptoms while working in the beehive and later developed respiratory allergic reactions to bee stings. The patient had

200 million years ago	first documentation of Hymenoptera as saprophages
150 million years ago	Hymenoptera become carnivores: the ovipositor transforms into the stinging apparatus
80 million years ago	first social, vegetarian Hymenoptera feeding from pollen and nectar: the honey bees
200 000 years ago	homo sapiens appears
8000 BC	conflict between bees and homo sapiens documented in a cave painting in Spain
2641 BC	Egyptian pharaoh Menes dies from the sting of a kheb, probably a wasp or a hornet
1699-1900	reports of unusual, systemic, sometimes fatal Hymenoptera sting reactions from Europe and US
1906	von Pirquet classifies systemic sting reactions as allergy
1925	Brown reports on successful immunotherapy with venom sac extract in a patient with bee venom allergy
1930	Benson introduces immunotherapy with whole-body extracts (WBE) from Hymenoptera, which becomes the routine procedure until 1979
1956	Mary Loveless treats three failures of WBE immunotherapy successfully with venom sac extracts
1965-75	diagnostic tests with WBE are far inferior to tests with venoms; reports of fatal sting reactions in patients on WBE immunotherapy
1978-79	two controlled prospective studies document the superiority of immunotherapy with venoms over WBE and placebo
1980	introduction of commercial Hymenoptera venoms for diagnosis and immunotherapy of patients with Hymenoptera venom allergy

Fig. 1: Timeline of important events in the history of Hymenoptera venom allergy.

positive skin tests to both bee venom sac and whole-body extracts and was successfully treated with bee whole-body extract (11). As a consequence, immunotherapy with whole-body extracts of Hymenoptera was introduced as a causal treatment and, based on several uncontrolled retrospective studies, considered to be very effective (12). In 1956, however, the American physician Mary Loveless described ten patients with severe allergic reactions to wasp stings, of whom three reacted severely after being re-stung during immunotherapy with whole-body extract (13). She went on to treat these

patients with venom sac extracts and of eight patients who were re-stung, none reacted again. However, in spite of these promising results, treatment with whole-body extracts continued for a further 23 years. It wasn't until the mid nineteen sixties that serious doubts about efficacy of whole-body extract for immunotherapy arose, following several reports of fatal sting reactions during this treatment (14). Also, skin tests using venoms and tests for the presence of venom-specific serum IgE were shown to be far more efficient at distinguishing between patients and controls than those using whole-body extracts (15, 16). Finally, two controlled prospective studies proved the superiority of immunotherapy with venom compared with whole-body extract and placebo, showing that whole-body extracts were no more effective than placebo. As a result, venom immunotherapy was introduced in 1980 and remains the standard causal treatment for insect sting allergy today (17, 18).

Disclosure

The author states no conflicts of interest.

References

1. Michener CD, Grimaldi DA. The oldest fossil bee: Apoid history, evolutionary stasis, and antiquity of social behaviour. Proc Natl Acad Sci USA 1988;**85**:6424-6426.
2. Urgeschichte der Menschheit. www.geschichte-schweiz/evolution-urmenschen.ch
3. Dams LR. Bees and honey hunting scenes in the mesolithic rock art of eastern Spain. Bee World 1978;**59**:45-53.
4. Cohen SG, Bianchine PJ. Hymenoptera, hypersensitivity, and history: A prologue to current day concepts and practices in the diagnosis, treatment and prevention of insect sting allergy. Ann Allergy 1995;**74**:198-217.
5. Staudigelius U. De curiosis post apum ictus symptomatibus. Miscellania curiosa sive ephemeridum medico - physicarum germanicarum Academiae caesareo-Leopoldinae. Naturae curiosorum Decuriae III 1699-1700;Observatio CXCIV: 314-18.
6. Desbret M. Observations sur les maladies épidémiques qui régnèrent à Cuffet et dans les environs: Avril. J Méd Chir Pharm 1765;**23**:153.
7. Portier P, Richet C. De l'action anaphylactique de certains venins. C.R. Soc Biol 1902;**54**:170-172.
8. Von Pirquet C. Allergie. Münch Med Wschr 1906:**53**:1457-1458.
9. Freeman J, Noon L. Further observations on the treatment of hayfever by hypodermic inoculations of pollen vaccine. Lancet 1911;**2**:814.
10. Braun LIB. Notes on desensitization of a patient hypersensitive to bee stings. S African Med Rec 1925;**23**:408-409.
11. Benson RL, Semenow AB. Allergy in first relation to bee sting. Allergy 1930;**1**:105-116.
12. Mueller HL, Hill LW. Allergic reactions to bee and wasp stings. New Engl J Med 1953;**249**:726-731.
13. Loveless MH, Fackler WR. Wasp venom allergy and immunity. Ann Allergy 1956;**14**:347-366.
14. Barnard JH. Studies on 400 Hymenoptera sting deaths in the United States. J Allergy Clin Immunol 1973;**52**:259-264.
15. Reisman RE, Wypych J, Arbesman CE. Stinging insect allergy: Detection and clinical significance of venom IgE antibodies. J Allergy Clin Immunol 1975;**56**:443-449.
16. Schwartz HJ. Skin sensitivity in insect allergy. JAMA 1965;**194**:113-115.
17. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. New Engl J Med 1978;**299**:157-61.
18. Müller U, Thurnheer U, Patrizzi R, Spiess J, Hoigné R. Immunotherapy in bee sting hypersensitivity. Bee venom versus wholebody extract. Allergy 1979;**34**:369-78.

PART ONE

Entomology, epidemiology, natural history and diagnostic tools

EPIDEMIOLOGY AND NATURAL HISTORY

M. Beatrice Bilò and Leonardo Antonicelli

Allergy Unit, Department of Internal Medicine, Allergy, Immunology and Respiratory Diseases, University Hospital, Ancona, Italy

Hymenoptera stings can induce an allergic and occasionally fatal reaction. The culprit Hymenoptera belong to the suborder Aculeate, which are made up of the Apoidea (*Apis mellifera, Bombus spp.*) and Vespidae (*Vespinae and Polistinae subfamilies*) superfamilies (1, 2). However, several of the European vespid species differ from those found in the USA where popular names for vespids are different and may lead to confusion (3). In the United States stinging Hymenoptera also include the fire ant (*Solenopsis invicta*), and in Australia, the jack jumper ant (*Myrmecia pilosula*) (1). The most frequent clinical manifestations of Hymenoptera venom allergy (HVA) are large local reactions (LLRs) and systemic reactions (SRs).

Venom sensitisation

The prevalence of venom sensitisation (indicated by a positive skin test and/or the detection of specific IgE in patients with no previous case history) in adults is estimated at between 9.3 and 28.7% (3, 4). Asymptomatic sensitisation occurs more commonly with repeated short-interval stings and is related to the degree of sting exposure (in 30-60% of beekeepers) as well as insect distribution and behaviour. Atopic individuals have an increased probability of testing positive for venom specific IgE. A genetic predisposition which enhances the production and/or the persistence of venom specific IgE and the presence of carbohydrate determinants (CCDs) in venoms and inhalant allergens which can cause cross-reactivity have been listed as possible causes. Detection of specific IgE to venoms in patients with no history of allergic sting reactions is often of no clinical relevance and may lead to a misdiagnosis (4). According to Golden et al. (5) the risk of subsequent sting-induced anaphylaxis in adults with asymptomatic venom sensitisation was 17%, while Fernandez et al. suggested that this condition is not a clear risk for a future SR, at least in the case of vespids (6). Nevertheless, asymptomatic venom sensitisation can, at least in part, explain the absence of a previous positive case history in 40% of fatalities. The time interval between stings may be one of the key factors in the switch from asymptomatic sensitisation to HVA.

Large local reactions

The prevalence of LLRs ranges from 2.4 to 26.4% in the general population, and up to 38% in beekeepers (3, 4). Skin tests and/or specific IgE are positive in up to 80% of patients with LLRs. Studies on small numbers of adults and children showed that patients with LLRs have a low risk (5-15%) of a SR when re-stung (4).

Table 1. Insect sting anaphylaxis: data extrapolated from the latest population-based studies of anaphylaxis due to any cause

Reference population (years)	Country	Type of study	Study population	Period of study (years)	No of cases	Insect sting (%)	No. of fatalities
Hebling, 2004	Switzerland	R	Adults	3	226	59	3*
Peng, 2004	UK	R	Children and adults	6	675	32	1**
Bohlke, 2004	USA	R	Children	6	67	22	0
Thong, 2005	Singapore	R	Adults	3.8	67	32.8	0
Mehl, 2005	Germany	R	Infants and Children	1	103	13	0
De Swert, 2008	Belgium	R	Children	2	64	7.3	0
Yang, 2008	Korea	R	Children and adults	6.6	138	11.8	1*
Decker, 2008	Rochester, Minnesota	R	Children and adults	10	211	18.5	0

R= retrospective P= Prospective
* Mortality not due to an insect sting
** Unknown cause of fatal anaphylaxis

Systemic reactions and anaphylaxis

In the United States, the prevalence of SRs to Hymenoptera sting in the general population ranges from 0.5 up to 3.3%. European epidemiological studies from the last decade report a prevalence of 0.3 to 8.9% for SRs, with anaphylaxis reported in 0.3 to 42.8% of cases (3, 4). The results of a small number of studies indicate that the prevalence rates of SRs are lower in children, ranging from 0.15% to 0.8%, with a recent exception of 4.4% reported in a study from Israel (7). Factors influencing the variability in the prevalence of SRs are described elsewhere (4). In children, about 60% of SRs are mild and generally cutaneous, whereas in adults about 70% of SRs are accompanied by respiratory or cardiovascular symptoms. In general, children have a better prognosis than adults with respect to the risk of SRs to re-stings; however, those who experience a moderate to severe SR continue to have a high risk of reactions even 15-20 years later (8). The risk of recurrence of SRs is linked to the severity of the previous reaction: the more serious the initial reaction, the greater the risk of recurrence. Linking the estimated risk of SR with severity, age, and sting interval, adults with a recent history of severe anaphylaxis have a 40 to 60% chance of reacting to a resting, whereas those with mild SRs have a 20% chance of a subsequent SR (9). The most recent population-based studies of anaphylaxis (all causes), reported that insect-sting-induced anaphylaxis was responsible for 7.3-59% of the total number of cases and was more frequent in adults than in children (3, 4) (Table 1). In a recent population-based retrospective study of the incidence and cause of anaphylaxis, conducted in Minnesota over a 10-year period from 1990 to 2000, insect stings accounted for 18.5% of

anaphylaxis cases (39 out of 211 cases) (10). Two studies from emergency departments (ED) of anaphylaxis due to any cause, found the prevalence of insect sting anaphylaxis during the last decade ranged from 1.5 to 34% (3, 4). The largest multicentre study of individuals who required ED treatment for insect sting allergic reactions was performed in 15 North American EDs. Of the total number of admissions, 358 (58%) were local reactions, while 259 (42%) were SRs; 27% of SRs were mild, 55% moderate, and 18% were severe (11).

Several factors may account for these varying estimates of the prevalence of insect sting-induced anaphylaxis, including, the lack of standard diagnostic guidelines, diverse population profiles, different International Classification of Disease-9 and other system codes, geography, and allergen exposure (4).

Biphasic anaphylactic reactions

Biphasic anaphylactic reaction (BAR) occurs in 1-20% of anaphylaxis cases and is generally associated with the most severe reactions; however, it is uncommon with insect stings (Table 2). Although fatalities are very rare in insect-sting BAR, but they can occur, raising the question of how long patients should be kept under observation following an anaphylactic reaction (12). Protracted infield insect sting anaphylaxis is extremely rare and delayed onset of anaphylaxis has been reported, but only anecdotally (4).

Table 2. Hymenoptera stings and biphasic anaphylactic reactions. Reproduced from Bilò BM, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. Clin Exp Allergy 2009;39:1467-76, with permission from Blackwell Publishing Ltd.

Reference population (years)	Country	Study population	Type of study	No. of biphasic reactions (%)	No. of fatalities
Bazil & MacNamara 1998	USA	Adults	ED	6 (18)§	0
Lee, 2000	Boston	Children	Hospital admissions	6 (6)**	0
Smit, 2005	Hong Kong	Children and adults	ED study	15 (5.3) *	0
Ellis, 2007	Canada	Children and adults	ED study	20 (19.4)^^	0
Yang, 2008	Korea	Children and adults	ED + inpatients + outpatients	3 (2.2)	0
De Swert, 2008	Belgium	Children	PB study	2 (3) *	0

PB study: population-based studies of anaphylaxis due to any cause
ED study: emergency department study of anaphylaxis due to any cause
§ Three cases due to an insect sting
* One case due to an insect sting
** Two cases due to bee stings
^^ Five cases due to an insect sting

Mortality
Mortality rates are low, but not negligible, the incidence of insect sting mortality due to early anaphylaxis, ranges from 0.03 to 0.48 fatalities per 1,000,000 persons per year (3, 4). Underestimates of insect sting mortality are common worldwide with many sting fatalities going unrecognised or misinterpreted and reported as unexplained.

Prevention of insect sting anaphylaxis
In 40-85% of fatal-sting reactions, victims had no history of previous anaphylaxis. This underlines the importance of developing a better understanding of risk factors, especially in asymptomatic sensitised subjects. In patients who experience a severe SR, survival often depends on the ability to rapidly access an ED and the correct use of epinephrine by the doctors. To prevent future SRs the patient should be referred to an allergist for diagnosis and, if appropriate, specific immunotherapy. Patients who are prescribed injectable epinephrine should be instructed in the proper technique for self-administration. However, most insect sting victims fail to seek medical advice and hospital attendance does not always correlate with the severity of the allergic reaction (11). A prompt and accurate diagnosis can identify those patients at greatest risk, and who would benefit from specific immunotherapy; as many as 50% of deaths in patients with a positive case history could have been avoided through the use of specific immunotherapy.
In contrast to the stable trends in age-specific admission rates for Hymenoptera stings, an Australian study reported an 88% decrease in the age-standardised rate for insect sting-induced fatal anaphylaxis over a 9-year period. According to the authors, possible explanations include the widespread use of insect venom immunotherapy and the increasing availability of adrenaline autoinjectors (13).

Conclusion
Despite the high prevalence of asymptomatic sensitisation, the prevalence of sting-induced anaphylaxis is comparably low. However, to date, no parameter has been identified that can predict the likelihood or severity of a future reaction and whether it will be local or systemic. The combination of several concomitant factors, including, environmental (the frequency of stings and the type of insect), genetic (the persistence of specific IgE antibodies and probably other factors), and individual factors (age; morbidities like asthma, mastocytosis, or ischaemic heart disease; concurrent medication; strenuous exercise; and others) may account for the occurrence of a SR in individual patients. In addition, several poorly characterised factors may be also be associated with the severity of a SR to re-stings. Better knowledge of the epidemiology, natural history and risk factors is crucial for improving the care of patients with insect venom allergies.

Disclosure
The authors state no conflicts of interest.

References
1. Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN; EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. Allergy 2005;**60**:1339-1349.
2. Muller UR. Insect sting allergy. Clinical picture, diagnosis and treatment. Stuttgart-New York;Gustav Fischer Verlag 1990.
3. Bilò BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. Curr Opin Allergy Clin Immunol 2008;**8**:330-337.
4. Bilò BM, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. Clin Exp Allergy 2009;**39**:1467-76.
5. Golden DB, Marsh DG, Freidhoff LR, Kwiterovich KA, Addison B, Kagey-Sobotka A et al. Natural history of Hymenoptera venom sensitivity in adults. J Allergy Clin Immunol 1997;**100**:760-766.
6. Fernandez J, Soriano V, Mayorga L, Mayor M. Natural history of Hymenoptera venom allergy in Eastern Spain. Clin Exp Allergy 2005;**35**:179-185.
7. Graif Y, Romano-Zelekha O, Livne I, Green MS, Shohat T. Allergic reactions to insect stings: results from a national sur-

vey of 10,000 junior high school children in Israel. J Allergy Clin Immunol 2006;**117**:1435-1439.
8. Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Outcomes of allergy to insect stings in children, with and without venom immunotherapy. N Engl J Med 2004;**351**:668-674.
9. Golden DB. Insect sting allergy and venom immunotherapy: a model and a mystery. J Allergy Clin Immunol 2005;**115**:439-447.
10. Decker WW, Campbell RL, Manivannan V, Luke A, St Sauver JL, Weaver A et al. The etiology and incidence of anaphylaxis in Rochester, Minnesota: a report from the Rochester Epidemiology Project. J Allergy Clin Immunol 2008;**122**:1161-1165.
11. Clark S, Long AA, Gaeta TJ, Camargo CA Jr. Multicenter study of emergency department visits for insect sting allergies. J Allergy Clin Immunol 2005;**116**:643-649.
12. Tole JW, Lieberman P. Biphasic anaphylaxis: review of incidence, clinical predictors, and observation recommendations. Immunol Allergy Clin North Am 2007;**27**:309-326.
13. Liew WK, Williamson E, Tang ML. Anaphylaxis fatalities and admissions in Australia. J Allergy Clin Immunol 2009;**123**:434-442.

RISK FACTORS IN HYMENOPTERA VENOM ALLERGY

Franziska Ruëff

Klinik und Poliklinik für Dermatologie und Allergologie, Ludwig-Maximilians-Universität, Munich, Germany

Current knowledge of risk factors must be considered when deciding which patients to treat with venom immunotherapy (VIT) and how to treat them. Risk factors may play a role in various aspects of Hymenoptera venom allergy, such as the severity of sting reaction, side effects, and inefficacy of VIT. Moreover, risk factors for systemic anaphylactic reactions (SAR) during VIT are not necessarily the same as those associated with severe field sting reactions or treatment failure; hence this is a subject that merits consideration and further research.

Sting reaction

The most important factor in the sensitisation to Hymenoptera venom is repeated exposure. Therefore, any activity that is associated with an increased risk of Hymenoptera stings will also increase the risk of sensitisation (Table 1). An increased risk of stings is even more important for those who have already had an anaphylactic sting reaction and are therefore disposed to a greater extent to future anaphylactic sting reactions. Venom sensitisation is a necessary, but not sufficient, prerequisite for subsequent systemic sting reactions. It is not fully understood why many sensitised individuals tolerate stings and others do not. The following risk factors are associated with a SAR: (*i*) history of a previous large local sting reaction (1), (*ii*) a short time interval to the last sting (1), and (*iii*) a higher concentration of venom-specific IgE in the serum (2). Conversely, a history of more than 200 stings per year seems to have a protective effect against anaphylactic sting reactions (3).

Another phenomenon is the varying occurrence of mild, moderate, or severe sting reactions in individuals and between different venom-allergic individuals. There are various classifications of the severity grade of a sting reaction. Here the classification of Ring and Meßmer (4) is used (Table 2). In brief, severe reactions are defined as anaphylactic shock (Grade III) or cardiopulmonary arrest (Grade IV). Several risk factors are associated with

Table 1. Increased exposure to Hymenoptera stings

Beekeepers and family members
Occupations such as: fruit seller, fireman, lumberjack, farmer, gardener
Outdoor activities, including gardening, picnicking, tennis, football, cycling
Beehives or wasps' nests close to living or working places

the occurrence of such severe systemic anaphylactic sting reactions in untreated subjects:

- Vespid stings are associated with more severe reactions than bee stings (5-7).
- An increase in baseline tryptase concentration from a value of 4.25 µg/L to 20 µg/L will augment the risk for a severe systemic reaction sting reaction by a factor of approximately 3.8 (7).
- Cutaneous (Figs. 1 and 2) and/or systemic mastocytosis (8, 9).
- Higher age (7).
- Angiotensin converting enzyme (ACE) inhibitors (7).
- Preceding, less severe systemic sting reactions seem to have a booster effect (5-7) and to dispose the patient to later severe reactions.

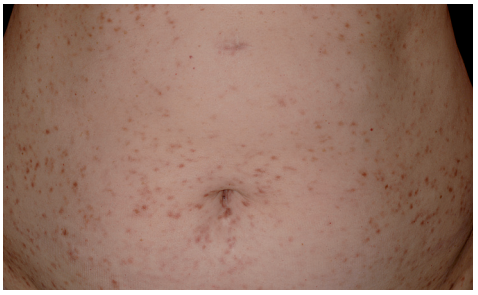


Fig. 1. Maculopapular cutaneous mastocytosis (Urticaria pigmentosa) in a 45-year-old female with indolent systemic mastocytosis

- The male subject predominance for severe reactions (5, 7) probably results from a selection effect. Due to a different degree of exposure, adult men are stung more frequently than women.

As a previous sting inducing only a mild reaction may act as a booster and may thus lead to more severe sting reactions in the future, it cannot be assured that the patient will have a good prognosis even if no risk factors are evident. However, for subjects with risk factors for later severe SAR to stings, VIT is particularly important. ACE inhibitors should be withdrawn in patients who are not treated and/or protected by VIT with certainty.

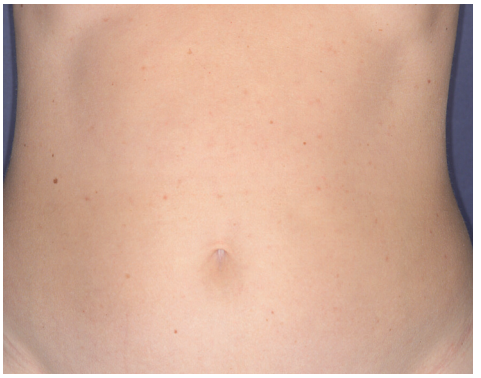


Fig. 2. Minor skin lesions in a 50-year-old female with indolent systemic mastocytosis

Side effects of VIT

VIT can be complicated by various side effects of which SAR are the most important. Large local reactions, unspecific systemic side effects like fatigue, or rare complications like serum sickness, are not being discussed here. In studies including a large number of patients 12% (10) up to 20% (11) of the patients exhibited SAR during VIT. The majority of these reactions were mild or moderate. If only moderate to severe reactions and reactions, which require medical treatment, are considered the percentage of affected patients is much lower, 6.7% (11) or 8.4% (12).

Several risk factors influence the overall risk

Table 2. Classification of systemic reactions modified according to Ring and Meßmer (4)

Grade I	Generalised skin symptoms (e.g. flushing, generalised urticaria, angioedema)
Grade II	Mild to moderate pulmonary, cardiovascular, and/or gastrointestinal symptoms
Grade III	Anaphylactic shock, loss of consciousness
Grade IV	Cardiac arrest, apnoea

of SAR and/or a particularly severe outcome of SAR during VIT:

- Treatment with honeybee venom induces SAR more frequently than treatment with vespid venoms (10-13).
- Side effects occur more often during the incremental phase of VIT than the maintenance phase (11).
- Tryptase concentration correlates significantly with the frequency of severe side effects during the dose-up phase of VIT (12); however, the extent of this effect depends on the type of venom and is stronger in patients receiving vespid VIT.
- Mastocytosis (9).
- Increasing age (12).
- Rapid dose-increase during dose-up phase (11, 12).
- During VIT, antihypertensive medications are associated with a higher risk for severe reactions (12). This finding suggests an association between pre-existing cardiovascular diseases and SAR during VIT. In this case, the discontinuation of respective medications before VIT will therefore probably have no beneficial effect. However, according to the current guidelines beta-blockers and ACE inhibitors should be discontinued before VIT is started (14). However, in the absence of information on the effects of a specific drug for SAR during VIT, more data on the association of antihypertensive medication and VIT are required before current recommendations can be revised. The decision as to whether these drugs should be withdrawn must also take into account the situation of the individual patient.

The severity of the sting reaction prior to treatment did not influence the risk for side effects (10-12). Regarding the risk factor associated with gender, the data are contradictory: when all SAR are considered, females had a higher incidence of SAR (11); if only more severe reactions were considered, females did not have an increased risk (12). Also, the presence of venom specific IgE in the serum was associated with a slightly higher risk for more severe SAR (12).
In conclusion, all patients undergoing VIT should be considered as candidates for SAR; risk factors must be taken into account and patients with one or more risk factor should be treated and monitored with special care.

Treatment failure

During VIT with the standard maintenance dose of 100 µg insect venom, treatment failure is not so rare. When sting challenges were performed in larger series of patients during the maintenance phase of VIT, up to 25% were not protected (15). An evaluation of 1071 of the author's own patients who underwent a sting challenge test revealed that SAR occurred in 7.7% of tests. To date, no multicentre studies of therapeutic efficacy that include a sufficient number of patients to enable a confounder-adjusted analysis of risk factors for SAR at sting challenge have been published. Nevertheless, several factors are currently known to predispose a patient to a SAR at sting challenge or a field sting:

- VIT with honeybee venom is less effective than treatment with vespid venom (13, 16, and author's unpublished data).
- There is a venom-specific effect of baseline tryptase concentration. Higher tryptase levels do not play a role for patients with honeybee venom allergy but have an effect for patients with vespid venom allergy (16).
- Venom dose; higher doses are more effective (15, 17).
- SAR during VIT (19 and author's unpublished data).
- Mastocytosis (9).

In a preliminary analysis of the author's own data, age, gender, and the severity grade of previous sting reactions were not predictive of SAR at sting challenge. Patients who are at risk of treatment failure and in whom reaction to a further sting may induce life-threatening symptoms should be offered treatment with an elevated maintenance dose of venom (200 µg or more) from the start of treatment. This is especially important in patients with honeybee venom allergy and one or more risk factors for treatment failure or a more severe anaphylactic reaction in untreated subjects (e.g., high sting exposure, mast cell disease).

Disclosure
The author states no conflicts of interest.

References

1. Pucci S, Antonicelli L, Bilò MB, Garritani MS, Bonifazi F. Shortness of interval between two stings as risk factor for developing Hymenoptera venom allergy. Allergy 1994;**49**:894-896.
2. Day JH, Buckeridge DL, Welsh AC. Risk assessment in determining systemic reactivity to honeybee stings in sting-threatened individuals. J Allergy Clin Immunol 1994;**93**:691-705.
3. Bousquet J, Ménardo JL, Aznar R, Robinet-Lévy M, Michel FB. Clinical and immunologic survey in beekeepers in relation to their sensitization. J Allergy Clin Immunol 1984;**73**:332-340.
4. Ring J, Meßmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977;**1**:466-469.
5. Lockey RF, Turkeltaub PC, Baird-Warren IA, Olive CA, Olive ES, Peppe BC et al. The Hymenoptera venom study I, 1979-1982: demographics and history-sting data. J Allergy Clin Immunol 1988;**82**:370-381.
6. Solley GO. Stinging and biting insect allergy: an Australian experience. Ann Allergy Asthma Immunol 2004;**93**:532-537.
7. Ruëff F, Przybilla B, Biló M-B, Müller U, Scheipl F, Aberer W et al. Predictors of severe systemic anaphylactic reactions in Hymenoptera venom allergy: the importance of baseline serum tryptase concentration and concurrent clinical variables. J Allergy Clin Immunol 2009;**124**:1047-1054.
8. Ruëff F, Dugas-Breit S, Przybilla B. Stinging Hymenoptera and mastocytosis. Curr Opin Allergy Clin Immunol 2009,**9**:338-342.
9. Niedoszytko M, de Monchy J, van Doormaal JJ, Jassem E, Oude Elberink JN. Mastocytosis and insect venom allergy: diagnosis, safety and efficacy of venom immunotherapy. Allergy 2009;**64**:1237-1245.
10. Lockey RF, Turkeltaub PC, Olive ES, Hubbard JM, Baird-Warren IA, Bukantz SC. The Hymenoptera venom study III: Safety of venom immunotherapy. J Allergy Clin Immunol 1990;**86**:775-780.
11. Mosbech H, Müller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. European Academy of Allergology and Clinical Immunology. Allergy 2000;**55**:1005-1010.
12. Ruëff F, Przybilla B, Biló M-B, Müller U, Scheipl F, Aberer W et al. Predictors of severe side effects during the build-up phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase concentration and other concurrent clinical variables. A study of the EAACI (European Academy of Allergology and Clinical Immunology) Interest Group on Insect Venom Hypersensitivity. Submitted.
13. Müller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. J Allergy Clin Immunol 1992;**89**:529-535.
14. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol 1998;**102**:558-562.
15. Ruëff F, Przybilla B, Müller U, Mosbech H. The sting challenge test in Hymenoptera venom allergy. Allergy 1996;**51**:216-225.
16. Haeberli G, Brönnimann M, Hunziker T, Müller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003;**33**:1216-1220.
17. Golden DBK, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Dose dependence of Hymenoptera venom immunotherapy. J Allergy Clin Immunol 1981;**67**:370-374.
18. Ruëff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving Hymenoptera venom immunotherapy are protected by increased venom doses. J Allergy and Clin Immunol 2001;**108**:1027-1032.
19. Müller U, Helbling A, Bischof M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. Allergy 1989;**44**:412-8.

DIAGNOSIS OF INSECT VENOM ALLERGY: SKIN TESTS AND SERUM-SPECIFIC IgE

M. Beatrice Bilò

Allergy Unit, Department of Internal Medicine, Allergy, Immunology and Respiratory Diseases, University Hospital, Ancona, Italy

The diagnosis of Hymenoptera venom allergy (HVA) is based on patient history, skin tests, and measurement of specific IgE antibodies (sIgE) in serum. Recent clinical practice parameters have been published by the European Academy of Allergology and Clinical Immunology (1) and by the American Academy of Allergy, Asthma and Immunology (2).

Indication for diagnostic testing

The indication for diagnostic testing is based on the clinical history of the patient. As venom sensitisation is found in around 10-20% of history-negative persons, only those with a history of a previous systemic reaction (SR) are candidates for diagnostic testing (1, 2) (Table 1). In some countries diagnostic tests are also performed in patients who have a history of large local reactions (LLR), even if they are considered as having a low risk of future SRs. Although individuals with a positive diagnostic test but negative history of a SR may be at risk of a future case of insect sting anaphylaxis, the current diagnostic tools cannot, as yet, be used for screening the general population (3).

Skin tests

Among skin tests, the venom skin prick test (SPT) and intradermal (ID) techniques are the methods of choice. It is recommended that skin tests be performed at least two weeks after a reaction to a sting to rule out false negative results during the refractory period (1). If the result is negative in the presence of a definitive history of a systemic sting reaction the test should be repeated after 1 to 2 months, as the duration of the refractory period may be longer (1). However, in some patients, sensitisation is demonstrable only in the first week after the sting (4). Commercial Hymenoptera venom products are available in many countries as lyophilised protein extract for honeybee, yellow jacket, and *Polistes* venoms, the latter two being mixtures of the clinically relevant species. Due to incomplete cross-reactivity between venoms of the European and American species of *Polistes* (5), commercial preparations of European *Polistes dominulus* venom have become available.

Stepwise incremental venom skin tests are recommended. If the patient has a conclusive reaction at a set concentration the test can be stopped. With the SPT, venom concentrations of 0.01 up to 100 µg/ml are usually used, while venom concentrations from 0.001 to 1 µg/ml are used for ID testing (1). Even at venom concentrations of 100 (g/ml, the sensitivity of the SPT is lower than that of the ID test. Therefore, it is recommended that an ID test be performed in patients with a negative SPT, to confirm the result. According to a number of studies, the sensitivity of ID testing is estimated to be 90% or higher at a venom concentration of 1 µg/ml

Table 1. Indications for diagnostic testing

Indication in:
- Patients with a history of a previous SR
- Patients with a history of a previous LLR (?)
No indication in:
- Subjects with a family history of a severe SR
- Subjects who have undue fear of developing a sting SR arising from a news report of a fatal sting reaction
- As a screening in the general population

SR: systemic reaction
LLR: large local reaction

(1) and results are more reproducible than those of the laboratory test for sIgE, formerly known as Radio Allergo Sorbent Test (RAST). The specificity of skin tests with Hymenoptera venoms is difficult to define, since exposed patients who never developed a systemic reaction may have become sensitised following their last sting (1). A study by Graif, in a small group of patients, showed that the reproducibility of venom skin tests and sIgE results was relatively poor and was not affected by the time elapsed between the SR and the first evaluation (6). Skin tests with Hymenoptera venoms are generally safe; nevertheless, SRs do occur very rarely (7).

Serum specific IgE antibodies

In vitro tests such as RAST and a variety of non-radioactive methods derived from this test can be applied to detect the presence of sIgE antibodies. The most recently developed tests are usually the most sensitive (1, 8). During the first few days after a sting, sIgE to the injected venom may be below the level of detection, increasing within days or weeks after a sting. Following this initial increase, sIgE declines slowly with a large variation between individuals (3). In patients with no detectable sIgE to the suspected venom, tests should be repeated after a few weeks (1). A rapid change of venom-specific IgE-antibodies shortly after a sting may provide an additional indication of the suspected venom (1), although skin testing is thought to be more specific than sIgE in such situation (9). Sensitivity of venom sIgE tests in patients with a history of systemic sting reactions is lower than that of intracutaneous skin tests, especially more than one year after a reaction (1). Similar problems to skin tests are encountered with regard to specificity.

Interpretation of skin test and *in vitro* test results

There is no positive correlation between the severity of previous sting reactions and skin test reactivity, or the concentration of venom sIgE (1). Indeed, the most reactive skin tests often occur in patients with only large local reactions, and in wasp venom allergy a significant inverse correlation has been demonstrated between the severity of sting reactions and sIgE concentrations (3). In a study that measured IgE antibody levels in sera from 51 victims of fatal venom anaphylaxis, IgE antibodies were not detected in 10% of the sera, and levels ranging from 0.35 to 1.1 ng/ml were found in 47%, indicating that fatal anaphylactic reactions to stings can occur in the presence of widely varying amounts of specific IgE antibody (10). In subjects with a history of a previous anaphylactic sting reaction, the demonstration of venom sensitisation by a skin test reaction to venom or the detection of venom-specific IgE-antibodies confirms sensitisation. To date, it has not been possible to find a marker that can predict the outcome of a re-sting, 25 to 84% of subjects with positive skin test reactions to venom do not react to a subsequent sting from the culprit insect, whereas up to 22% of subjects with negative skin tests develop a systemic reaction (1, 2).

Double sensitisation with a single history

Double positivity to both bee and *vespid* venoms in diagnostic tests is observed in 25-40% of patients with insect allergy. The majority of these patients have a single-positive history and many of them are unable to identify the culprit insect. Double positivity may be due to double sensitisation, cross-reactivity between epitopes on hyaluronidase in the two venoms (1), or to cross-reactivity between carbohydrate epitopes (CCDs) of venoms and common allergens (11). Any of these may induce multiple positive test results which have unknown clinical significance. The IgE-inhibition test is helpful in distinguishing between cross-reactivity and double sensitisation, however, it is costly and results are sometimes difficult to interpret. In a recent study that included 200 patients with a history of reaction to honeybee or *Vespula* sting, 59% of allergic patients showed double positivity for specific IgE using the ImmunoCAP® test, and 32% of patients had double positivity using ADVIA Centaur® (12). Specific IgE to the recombinant non-glycosylated major allergen phospholipase A2 (Api m 1) were present in 99% of whole bee venom-positive allergic patients, while sIgE to antigen 5 (Ves v 5) were present in 96% of whole *Vespula* venom-positive allergic patients when tested by ADVIA Centaur®. Thus, the double positivity was reduced to 17%. The authors concluded that this approach could reduce the treatment costs in patients with double positivity by improving the identification of venoms for immunotherapy (12).

"Negative" test results

The prevalence of negative results in skin tests and sIgE among patients who seek medical care for sting-induced allergic reactions or those recruited for clinical trials ranges from less than 10% to more than 20%. Negative test results can be due to the involvement of a different pathogenetic mechanism, the presence of mast cell disease, the elapsed time since the last reaction, or insufficient sensitivity of tests. The failure to detect venom sIgE, however, provides no guarantee of clinically reduced reactivity. Among 99 patients with negative skin test results, the sting challenge was positive in 24% of the patients with positive sIgE (using a very sensitive technique performed at the John Hopkins School laboratory) and in 14% of patients with a negative sIgE test (13), underlying the need to perform both skin and sIgE tests. There has been one report that the ImmunoCAP® test detected a concentration of sIgE between 0.1 and 0.35 kU/L in a few patients with a positive history of yellow jacket allergy and negative skin and sIgE results (14). However, further studies are needed in order to confirm the utility of this modified assay in venom allergy.

In some patients, negative ID test results with commercial un-dialysed venoms (UV) can be clinically misleading. A recent study showed that in these cases, yellow jacket allergic sensitisation can usually be detected using a skin test with dialysed venom (DV) (15). Skin test results to 10 (g/ml or less of DV were positive in 79% of patients with a positive history, but were negative to UV and three of these patients experienced a SR to sting challenge (15). Negative test results can also result from failure to use the correct venom for the test. Studies have demonstrated partial cross-reactivity among venoms of the European (*dominulus* and *gallicus*) and the American (*exclamans, annularis fuscatus*) species of *Polistes* (5), emphasising the need to choose the correct venom for both diagnostic and therapeutic purposes (16). It is well known that *Vespa crabro* (VC) sting is a risk factor for severe SR (1). VC venom possesses some antigens in common with *Vespula* venom, however, one third of VC-allergic individuals have positive skin and sIgE tests restricted to VC venom. Therefore, depending on the geographic location, all relevant venoms should be tested. Finally, additional *in vitro* tests (such as the basophil activation test) can be used to demonstrate immunologic sensitisation in patients that are difficult to diagnose, however, their use should be restricted to laboratories with expertise in performing these tests.

Diagnosis of Hymenoptera venom allergy (HVA) in "real life"

Recently, the first comprehensive audit of the diagnosis and management of patients with HVA in the UK showed that 55% of specialists use serum sIgE as the first test for HVA (17). The remainder use a SPT rather than ID test, and 50% of specialists use an ID test in patients with a history of a severe SR if the SPT and sIgE are negative. When ID is undertaken, 40% of practitioners used concentrations 10 times higher than that recommended by the guidelines, giving a likelihood of false positive results. When skin tests were negative, despite a positive clinical history of SR, only 46% of the clinicians repeated skin tests along with sIgE after an interval of 1-2 months (17). It remains to be seen, however, to what extent the UK situation can be extrapolated to other European countries.

Conclusions

Although recent European and American practice parameters have been published on insect venom diagnosis, a recent UK study demonstrates a wide variation in practice and suggests a need for the development of better educational programmes for specialists and trainees involved in the management of patients with HVA. Further studies from other countries are needed in order to evaluate the application of the guidelines in the current clinical practice.

Optimised diagnostic procedures are needed, not only to detect IgE-mediated sensitisation to Hymenoptera venom, but also to predict clinical reactivity, identify the offending insect, and evalute the risks for the individual patient. In order to achieve these goals practitioners must take into account the patient's history, repeat skin tests and measurement of sIgE and, if needed, supplement these by additional *in vitro* diagnostic tests.

Disclosure

The author states no conflicts of interest.

References

1. Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN; EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. Allergy 2005;**60**:1339-1349.
2. Moffitt JE, Golden DB, Reisman RE, Lee R, Nicklas R, Freeman T et al. Stinging insect hypersensitivity: a practice parameter update. J Allergy Clin Immunol 2004;**114**:869-86.
3. Golden DK. Diagnostic methods in insect allergy. In: Monograph on insect allergy. Fourth edition. Eds Levine MC and Lockey RF, 2003. p. 63-74.
4. Goldberg A, Confino-Cohen R. Timing of venom skin tests and IgE determinations after insect sting anaphylaxis. J Allergy Clin Immunol 1997;**100**:182-184.
5. Pantera B, Hoffman DR, Carresi L, Cappugi G, Turillazzi S, Manao G et al. Characterization of the major allergens purified from the venom of the paper wasp Polistes gallicus. Biochim Biophys Acta 2003;**1623**:72-81.
6. Graif Y, Confino-Cohen R, Goldberg A. Reproducibility of skin testing and serum venom specific IgE in Hymenoptera venom allergy. Ann Allergy Asthma Immunol 2006;**96**:24-29.
7. Muller UR. Insect sting allergy. Clinical picture, diagnosis and treatment. Stuttgart-New York;Gustav Fischer Verlag 1990.
8. Hamilton RG. Diagnostic methods for insect sting allergy. Curr Opin Allergy Clin Immunol 2004;**4**:297-306.
9. Egner W, Ward C, Brown DL, Ewan PW. The frequency and clinical significance of specific IgE to both wasp (Vespula) and honey-bee (Apis) venoms in the same patient. Clin Exp Allergy 1998;**28**:26-34.
10. Hoffman DR. Fatal reactions to hymenoptera stings. Allergy Asthma Proc 2003;**24**:123-7.
11. Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhrl S et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol 2001;**108**:1045-52.

12. Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honeybee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy 2009;**64**:543-548.
13. Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Insect sting allergy with negative venom skin test responses. J Allergy Clin Immunol 2001;**107**:897-901.
14. Guerti K, Bridts CH, Stevens WJ, Ebo DG. Wasp venom-specific IgE: towards a new decision threshold? J Investig Allergol Clin Immunol 2008;**18**:321-323.
15. Golden DB, Kelly D, Hamilton RG, Wang NY, Kagey-Sobotka A. Dialyzed venom skin tests for identifying yellow jacket-allergic patients not detected using standard venom.Ann Allergy Asthma Immunol 2009;**102**:47-50.
16. Bilò MB, Brianzoni F, Cinti B, Napoli G, Bonifazi F. The dilemma of the negative skin test reactors with a history of venom anaphylaxis: will this always be the case? Eur Ann Allergy Clin Immunol 2005;**37**:341-342.
17. Diwakar L, Noorani S, Huissoon AP, Frew AJ, Krishna MT. Practice of venom immunotherapy in the United Kingdom: a national audit and review of literature. Clin Exp Allergy 2008;**38**:1651-1658.

ADDITIONAL *IN VITRO* TESTS IN THE DIAGNOSIS OF HYMENOPTERA VENOM ALLERGY

Franziska Ruëff

Klinik und Poliklinik für Dermatologie und Allergologie, Ludwig-Maximilians-Universität, Munich, Germany

In patients with a history of anaphylactic sting reactions, *in vitro* tests are performed in order to demonstrate sensitisation to the causative venom. However, the diagnosis is sometimes problematic: either the patient has no venom-specific IgE antibodies (v-sIgE), or sIgE only to a presumably false venom; or the patient is sensitised to various venoms despite having a history of only one sting reaction. Test methods for v-sIgE antibodies are presented elsewhere in this volume, (Diagnosis of insect venom allergy), and the relevance of cross-reactivity between vespid and honeybee venom by specific IgE inhibition is also discussed (Cross-reactivity in venom allergy: Clinical Relevance). Thus, herein the focus is on other tests.

IgE-negative patients with a history of an anaphylactic sting reaction

In the case of patients that lack v-sIgE in the serum, the different sensitivity of the various commercial test systems should be the first consideration. It is possible that a more sensitive test can detect sIgE where another has failed. The sensitivity of the immunoblot test is superior to commercial ELISA tests, enabling it to identify v-sIgE to individual venom allergens (1). Another important aspect is the time of serum sampling. Some patients may have v-sIgE within the first weeks after the sting reaction (2), while others develop v-sIgE only several weeks after the reaction (2, 3). Thus, in the case that a test fails to identify v-sIgE shortly after the sting, it is useful to perform another test after several weeks.

On average the serum concentration of v-sIgE declines steadily after a sting (4); in some patients, v-sIgE may be no longer demonstrable several weeks after the sting, although this usually occurs after several months to years. Negative tests (skin test, v-sIgE) in patients with a history of an anaphylactic sting reaction are not a reliable indicator of the patient's state of clinical tolerance to insect stings (5). Thus, in patients with a history of severe sting reaction or other risk factors, cellular tests should be tried in order to demonstrate venom sensitisation. However, due to the high technical complexity of cellular tests these should only be performed in specialised laboratories and results interpreted by experienced staff (6).

- In the basophil histamine release test, peripheral blood leucocytes are incubated with venom allergens, which react with cell-bound v-sIgE antibodies and thus stimulate basophils to release histamine. Studies have demonstrated the sensitivity of the histamine release to be between 50 and 90% (7, 8).
- In the leukotriene release test, or cellular antigen stimulation test (CAST), blood leucocytes are pre-stimulated with IL-3 and

exposed to venom allergens. The resulting release of sulfidoleukotrienes is determined by ELISA (7). CAST sensitivity has been reported to be between 83 and 100% (7, 8).
- The basophil activation test (BAT) is based on the flow cytometric demonstration of an altered membrane phenotype of activated basophils. Currently, the most commonly used marker to demonstrate basophil activation is CD63. However, CD203c may also be up-regulated and has been found to be slightly more sensitive than CD63 (9). The sensitivity of the BAT measuring the CD63 expression was 90 to 100 % (8-11).

In patients with a history of an allergic reaction to honeybee or vespid sting, the different venoms show different activation potential. This leads to different sensitivity of the cellular tests depending on the type of venom used (7). Differences in the receptors present on the surface of basophils may play a role in determining the sensitivity of cellular tests that measure basophil function upon allergen stimulation. The individual reactivity of basophils to allergens shows a high variability and some do not react and basophils from a minority of venom-allergic subjects do not release histamine in the presence of antigen (12). Furthermore, the results of different cellular tests do not always agree, patients may respond to one cellular test and not to an other (7, 8, 13). Thus, it is recommended that various tests be performed if the demonstration of venom sensitisation is important for the patient.

Double sensitisation in patients with a history of one anaphylactic sting reaction

Despite many efforts, it has thus far not been possible to find any laboratory marker that indicates more than sensitisation. Thus, the future systemic reactivity of the individual patient cannot be predicted from any test, and at present, cellular tests cannot be regarded as superior to measurement of sIgE. In patients with a history of only one sting reaction who show sensitisation to more than one venom, two other test methods may give a clue to the identity of the causative insect:
- Change of v-sIgE after a sting reaction. The sting acts as a booster and will increase the concentration v-sIgE to the insect responsible, whereas v-sIgE to insects, to which the patient was not exposed, will not change or show only minor increase due to cross-reacting antibodies (3). In some patients this characteristic change of v-sIgE after the sting may sometimes identify the culprit venom (see Fig. 1). This is usually the case if blood samples can be repeatedly taken in the first days or weeks after the sting and if no other stings have occurred since.

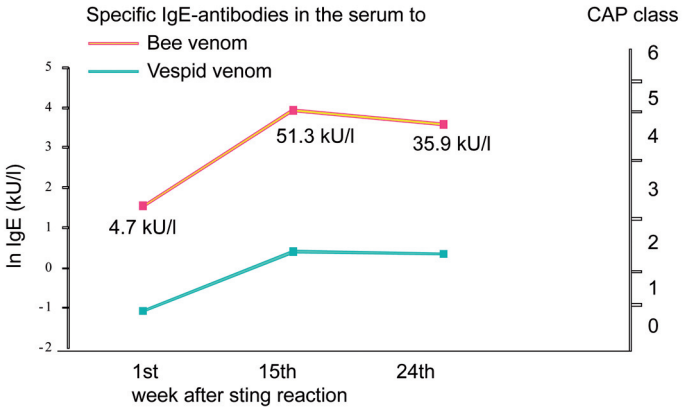


Fig. 1. Specific IgE antibodies in the serum of a 34-year-old female reporting severe anaphylaxis after "wasp sting". The concentration of bee-venom-specific IgE antibodies markedly increased after the sting, indicating that a honeybee and not a wasp was responsible for the sting.

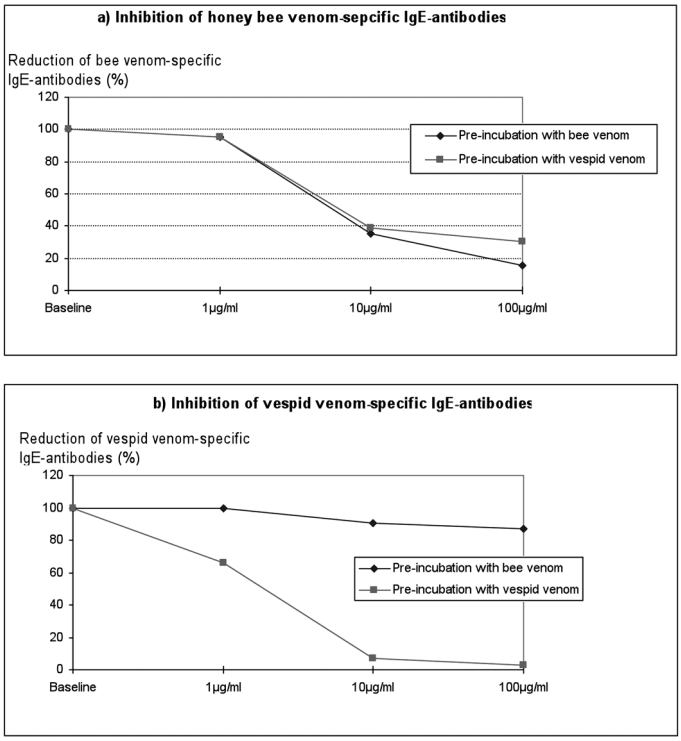


Fig. 2. Skin test reaction to 100 µg/ml of both yellow jacket and honeybee venom, as well as to various aeroallergens following a moderate systemic reaction to a sting in the neck. Specific IgE is demonstrable in CAP-class II to honeybee (2.27 kU/L) and vespid (2.24 kU/L) venom. ImmunoCAP(r)-inhibition shows a clear dose-dependent decrease in bee venom-specific IgE following pre-incubation with both bee and vespid venom (a). There was no decrease in vespid v-sIgE after pre-incubation with honeybee venom (b), but only with vespid venom, which acts as a control. Venom immunotherapy was performed with vespid venom.

- sIgE inhibition aims to demonstrate cross-reactivity between venoms or between venoms and other sources of allergen, e.g. rapeseed pollen (14). By pre-incubation of the patient's serum with venom in various concentrations, the respective v-sIgE is bound and therefore not able to bind to venom allergens in the commercial test system. v-sIgE in the pre-treated sample is then measured by a sIgE test (14). If, for example, the pre-incubation with honeybee venom reduces not only the detection of honeybee v-sIgE but also vespid v-sIgE, the test indicates cross-reactivity. In individual patients the demonstration of such cross-reactivity may make it easier to perform treatment with a single venom, despite double sensitisation (see Fig. 2).

Despite having a history of only one sting the patient may have "true" double sensitisation. In that case, the patient must be treated with several insect venoms.

Risk assessment: Serum tryptase

An elevated baseline serum tryptase level is a risk factor for particularly severe sting reactions, as well as side effects and treatment failure of VIT. This mainly occurs in vespid venom-allergic patients (see chapter on Risk Factors in this volume), and should be determined in all patients with Hymenoptera venom allergy at least in those with moderate to severe reactions (15).

Disclosure

The author states no conflicts of interest.

References

1. Zollner TM, Spengler K, Podda M, Ergezinger K, Kaufmann R, Boehncke WH. The Western blot is a highly sensitive and efficient technique in diagnosing allergy to wasp venom. Clin Exp Allergy 2001;**31**:1754-1761.
2. Goldberg A, Confino-Cohen R. Timing of venom skin tests and IgE determinations after insect sting anaphylaxis. J Allergy Clin Immunol 1997;**100**:182-184.
3. Rieger-Ziegler V, Rieger E, Kränke B, Aberer W. Hymenoptera venom allergy: time course of specific IgE concentrations during the first weeks after a sting. Int Arch Allergy Immunol 1999;**120**:166-168.
4. Mosbech H, Christensen J, Dirksen A, Søborg M. Insect allergy. Predictive value of diagnostic tests: a three-year follow-up study. Clin Allergy 1986;**16**:433-440.
5. Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Insect sting allergy with negative venom skin test responses. J Allergy Clin Immunol 2001;**107**:897-901.
6. Kleine-Tebbe J, Erdmann S, Knol EF, MacGlashan DW Jr, Poulsen LK, Gibbs BF. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. Int Arch Allergy Immunol 2006;**141**:79-90.
7. Maly FE, Marti-Wyss S, Blumer S, Cuhat-Stark I, Wüthrich B. Mononuclear blood cell sulfidoleukotriene generation in the presence of interleukin-3 and whole blood histamine release in honey bee and yellow jacket venom allergy. J Investig Allergol Clin Immunol 1997;**7**:217-24.
8. Sainte-Laudy J, Sabbah A, Drouet M, Lauret MG, Loiry M. Diagnosis of venom allergy by flow cytometry. Correlation with clinical history, skin tests, specific IgE, histamine and leukotriene C4 release. Clin Exp Allergy 2000;**30**:1166-71.
9. Eberlein-König B, Varga R, Mempel M, Darsow U, Behrendt H, Ring J. Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. Allergy 2006;**61**:1084-1085.
10. Erdmann SM, Sachs B, Kwiecien R, Moll-Slodowy S, Sauer I, Merk HF. The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. Allergy 2004;**59**:1102-1109.
11. Ebo DG, Hagendorens MM, Schuerwegh AJ, Beirens LM, Bridts CH, De Clerck LS et al. Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. Cytometry B Clin Cytom 2007;**72**:196-203.
12. Radermecker MF, Leclercq MD, Mariz SD, Louis RE. Basophil releasability in patients with hymenoptera venom allergy. Int Arch Allergy Immunol. 1993;**101**:283-287.
13. Eberlein-König B, Rakoski J, Behrendt H, Ring J. Use of CD63 expression as marker of in vitro basophil activation in identifying the culprit in insect venom allergy. J Investig Allergol Clin Immunol 2004;**14**:10-16.
14. Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhrl S et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol 2001;**108**:1045-1052.
15. Biló BM, Ruëff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy 2005;**60**:1339-1349

HYMENOPTERA VENOMS: COMPOSITION, STANDARDISATION AND STABILITY

Markus Ollert

Clinical Research Division of Molecular and Clinical Allergotoxicology, Department of Dermatology and Allergy, Technische Universität München (TUM), Munich, Germany

Hymenoptera venoms are important sources of allergens that lead to clinically relevant systemic allergic reactions. In Europe, hymenoptera venom allergy is caused by stinging insects of the *Apidae* and *Vespidae* families. Amongst the *Apidae*, most stings leading to an allergic reaction are caused by honeybees (*Apis mellifera*) and only a minority by bumble bees or other wild bees. Amongst the *Vespidae*, *Vespula spp.* are responsible for the majority of allergic reactions in Northern and Central Europe, where they are the most prevalent species, whereas in milder climates such as the Mediterranean *Polistes spp.* prevail. In addition, hymenoptera venom allergy can also be caused, although to a lesser extent, by stings from other members of the *Vespidae* family such as the European hornet (*Vespa crabro*) or *Dolichovespula spp.*

Hymenoptera venom composition and elicitors of venom allergy

Hymenoptera venoms are composed of low molecular weight components, including amines and toxins, and higher molecular weight proteinaceous constituents. The lower molecular weight compounds, such as histamine, dopamine, and phospholipids, are not allergenic but may be involved in sensitisation. Venom allergens are commonly found within the higher molecular weight fraction of peptides and proteins, many of which have enzymatic activity. Tables 1 and 2 summarise the important structural features and relative content in whole dried venom of the currently known allergens in *Apis mellifera* and in *Vespula vulgaris* venom. In bee venom, the only allergenic peptide is melittin, which has a molecular weight of around 3 kDa, but can also exist as a larger oligomer. The other important allergenic molecules are large proteins, with the most abundant being the 17 kDa phospholipase A2 (Table 1). The major allergens in vespid venom, have a similar molecular weight to those found in bee venom, with Antigen 5 being the dominant allergen. The allergen composition between different venoms from the *Vespidae* family is very similar, and thus there is a high degree of IgE cross-reactivity between genera such as *Vespula*, *Vespa*, and *Dolichovespula* due to the conserved molecular characteristics of the allergens. In contrast, the venom of *Polistes spp.* displays an allergen pattern that can be differentiated from the other genera of the *Vespidae* family. IgE cross-reactivity can also occur between the venoms of some members of the *Apidae* and *Vespidae* families if IgE binding is directed against cross-reactive allergens such as the hyaluronidases (Api m 2, Ves v 2) or the dipeptidyl peptidases (Api m 5, Ves v 3), which have more than 50% amino acid sequence homology.

Table 1. Allergens in *Apis mellifera* venom.

Allergen	Common name/ Function	MW	Potential N-Glycosylation sites	% of dry venom
Api m 1	Phospholipase A2	17 kDa	1	10-12%
Api m 2	Hyaluronidase	45 kDa	2	1-3%
Api m 3	Acid Phosphatase	49 kDa	2-3	1%
Api m 4	Melittin	3 kDa	0	50%
Api m 5	Allergen C / DPP IV	100 kDa	5-7	1%
Api m 6	Protease-Inhibitor?	8 kDa	0	1-2%
Api m 7	CUB-Protease	39 kDa	2-4	?
Api m 8	Carboxylesterase	70 kDa	4	?
Icarapin	Venom protein 2	55 kDa	3	?
VP 58		58 kDa	3	?
VP 60		60 kDa	4-5	?
VP 200		200 kDa	1-2	?

Hymenoptera venom products for patient use

The stability of venom products for diagnostic use is an important quality; in general, honeybee venom is much more stable than venom from vespids. In the lyophilised form, with added human serum albumin, most venom fractions remain stable for up to several years, however, once the venom is in solution stability is much lower. The standardisation of venom extracts for use in diagnosis and immunotherapy is also important. The quality of venom products can now be tested using a range of immunoassays, such as IgE inhibition and human recombinant IgE antibodies directed at specific epitopes on the allergens. For allergens with enzymatic activity, it is possible to test if this is present.

Recombinant allergens

One way to improve the standardisation of allergens for diagnostic use, and also for the development of quality controls for venom extracts, is through the use recombinant allergens. Although recombinant Hymenoptera allergens can be expressed in a variety of cell types, the use of different cell lines can result in varying amounts of cross-reactive carbohydrate determinants (CCDs) on the expressed allergen. Levels of CCDs on Api m 5 are reduced by expression in mammalian and plant cells compared with insect cells, however, the protein epitope remains intact. As CCDs are an important feature of the allergen, the use of a eukaryotic baclovirus expression system is recommended to ensure correct folding and glycosylation of the expressed protein. The enzymatic activity of the Api m 4 and Api m 5 allergens from *Apis mellifera* venom was preserved when the allergens were expressed in eukaryotic baclovirus system, but not in *E. coli* or other prokaryotic expression systems. In addition, recombinant allergens can be used to produce recombinant monoclonal antibodies that can be used for the production and quality control of allergens and also for ELISA-based laboratory assays. Recombinant IgG4 antibodies generated against Api m 1, 3, 5 and Ves v 5 were able to detect the natural allergens in venom extracts.

Table 2. Allergens in *Vespula vulgaris* venom.

Allergen	Common name/ Function	MW	Potential N-Glycosylation sites	% of dry venom
Ves v 1	Phospholipase A1	35 kDa	0	6-14%
Ves v 2a	Hyaluronidase	42 kDa	2-4	1-3%
Ves v 2b	Hyaluronidase	42 kDa	2	1-3%
Ves v 3	DPPIV	100 kDa	3	1%
Ves v 4	CUB-Protease	42 kDa	2-4	?
Ves v 5	Antigen 5	25 kDa	0	5-10%
Ves v 200 kd		200 kDa	?	?

Future perspectives

Based on the new molecular information and recent technological advancements, a paradigm shift in the standardisation of hymenoptera venom allergens for diagnosis and treatment is already feasible today. However, the future standardisation of venoms for diagnosis and treatment should not only focus on the abundant major allergens such as phospholipase A2 (Api m 1) in honeybee venom, or antigen 5 (Ves v 5) in vespid venom, but also on less abundantly expressed allergens such as Api m 3 and Ves v 3. Our increasing knowledge of the molecular allergen content of Hymenoptera venoms and the potential use of recombinant hymenoptera venom allergens in diagnosis, will not only lead to new and improved diagnosis and treatment of Hymenoptera venom allergy, but also to improved quality control of the diagnostic and therapeutic agents.

Disclosure

The author states no conflicts of interest.

References

1. Seismann H, Blank S, Cifuentes L, Braren I, Bredehorst R, Grunwald T, et al. Recombinant phospholipase A1 (Ves v 1) from yellow jacket venom for improved diagnosis of hymenoptera venom hypersensitivity. Clin Mol Allergy 2010;**8**:7.
2. Blank S, Seismann H, Bockisch B, Braren I, Cifuentes L, McIntyre M, et al. Identification, Recombinant Expression, and Characterization of the 100 kDa High Molecular Weight Hymenoptera Venom Allergens Api m 5 and Ves v 3. J Immunol 2010 Mar 26 [Epub ahead of print]
3. Seismann H, Blank S, Braren I, Greunke K, Cifuentes L, Grunwald T, et al. Dissecting cross-reactivity in hymenoptera venom allergy by circumvention of alpha-1,3-core fucosylation. Mol Immunol 2010;**47**:799-808.

CROSS-REACTIVITY IN VENOM ALLERGY: CLINICAL RELEVANCE

Wolfgang Hemmer

FAZ - Floridsdorfer Allergiezentrum, Vienna, Austria

Simultaneous reactivity to more than one Hymenoptera venom is commonly observed in patients with hypersensitivity to stinging insects. Multiple reactivity may be caused by stings from more than one insect, or by cross-reactivity. Understanding the sensitization pattern by exploring cross-reactions and identifying the primary venom is essential for selection of the correct vaccine for specific immunotherapy and in estimating the risk of a reaction to sting of a different species.

Cross-reactivity between honeybee and *Vespula* venom

Double sensitisation to honeybee and yellow jacket (*Vespula*) venom is encountered in 30-60% of patients with insect venom allergy (1-5). According to studies using inhibition experiments or species-specific recombinant allergens, true double sensitisation is responsible for about 20-40% of double-positive results whereas the remaining cases result from cross-reactivity (3, 4, 6-8). Fucosylated N-glycans (cross-reactive carbohydrate determinants; CCDs) account for 50-60% of all double-positive results (6-8). Yellow jacket venoms contain a single established glycoallergen, hyaluronidase (Ves v 2) (Table 1), whereas in honeybee venom most major and minor allergens, including phospholipase A2, hyaluronidase, protease, and acid phosphatase, are glycosylated. As a consequence, CCD-positive sera may show particularly high *in vitro* scores for honeybee venom. CCD-specific IgE antibodies have no or very limited clinical relevance, therefore, positive results due to CCD cross-reactions should be considered as false positive.

Protein-specific cross-reactivity between honeybee and yellow jacket venom is primarily caused by the venom hyaluronidases (Api m 2/Ves v 2), which have around 50% sequence identity. Since most of the IgE against hyaluronidases is directed against attached N-glycans, protein-induced cross-reactivity is now estimated to be rare (9-10). Cross-reactions to dipeptidylpeptidase IV (Api m 5/Ves v 3), which is another allergen shared by honeybee and yellow jacket venom, do not appear to be a major cause for double-positivity (9). Protein-specific cross-reactivity between venom hyaluronidases is commonly assumed clinically relevant, but at present this is not well supported by experimental data. It is largely unknown whether rare cases of anaphylaxis occurring after stings from both bees and wasps in a single individual, comes from true double sensitization or from cross-reactivity (1, 2). A careful evaluation of such patients is required for a better insight into the potential significance of hyaluronidase cross-reactions.

The classical method used to distinguish between true double sensitisation and cross-reactivity is reciprocal IgE inhibition (4, 5).

Table 1. Allergen sequence similarity of *Vespula* allergens

***Vespula vulgaris* allergen**	**Sequence identity of homologous proteins in other *Vespula* species**	
PLA1 **Ves v 1** (P49369)	*Vespula maculifrons* (P51528) *Vespula germanica* (Q3ZU95) *Vespula squamosa* (Kolarich 2007)	95% 94% 71%
Hyaluronidase **Ves v 2** (P9370)	*Vespula germanica* (Q05FZ2):	99%
Antigen 5 **Ves v 5** (Q05110)	*Vespula flavopilosa* (P35783) *Vespula maculifrons* (P35760, Q2L6Z1) *Vespula germanica* (Q3ZU96) *Vespula pensylvanica* (P35785) *Vespula vidua* (P35787) *Vespula squamosa* (P36786)	98% 92-95% 94% 93% 74% 71%

Recent data has emphasised the importance of including an appropriate CCD-inhibitor to reliably identify cross-reactions caused by CCD alone (6-8). Screening for anti-CCD IgE antibodies in individual sera using commercial assays (e.g. specific IgE test against bromelain) may be helpful in identifying CCD-positive patients; however, positive results do not reliably exclude true double-sensitisation, since around 10-20% of CCD-positive sera simultaneously contain IgE against unique peptide epitopes in both venoms. These CCD-related problems should be overcome when commercial assays using recombinant venom allergens become available.

Cross-reactivity within the *Apidae* family (bees and bumblebees)

The allergens from different honeybee species (*Apis mellifera, A. cerana, A. dorsata*) are virtually identical, with sequence identities of major and minor allergens always exceeding 90%. In allergy tests, most honeybee venom-allergic subjects cross-react to a certain extent with bumblebee venom (*Bombus spp.*), which presumably explains allergic reactions to occasional bumblebee stings in honeybee-allergic patients (11). Remarkably, patients with a genuine sensitisation to bumblebee venom (e.g. workers heavily exposed to bumblebees used as pollinators in greenhouses) showed only moderate cross-reactivity with honeybee venom (12). Thus, immunotherapy using honeybee venom may be ineffective in such patients (13). Consistent with these findings, recent molecular data indicated only 53% identity between the phospholipases from the two venoms (11).

Cross-reactivity between wasp venoms (*Vespidae*)

The allergens of different *Vespula* species have very high sequence identity with each other (around 95%) and are largely immunologically indistinguishable (Table 2) (14, 15). Conspicuous exceptions are the American species *V. squamosa* and *V. vidua*, both of which show significantly reduced cross-reactivity with the other members of the same genus. While venoms from different *Vespula* species are considered to be equivalent and interchangeable with regard to allergy diagnosis and immunotherapy, controlled sting challenges show a significantly higher rate of systemic reactions to *Vespula maculifrons* specimens as compared with *V. germanica* (16).

There is also evidence of substantial cross-reactivity of *Vespula* venoms with those from other *Vespinae*, including *Dolichovespula spp.* and *Vespa crabro*. (European hornet), even though restricted reactivity with single venoms has been observed with mouse and

Table 2. Allergen sequence similarity of *Vespinae* and *Polistinae* allergens.

a5	*Vespula*	-	*Dolichovespula, Vespa*	64-73%
	Vespula	-	*Polistes*	57-59%
	Polistes	-	*Polistes*	85-98%
PLA1	*Vespula*	-	*Dolichovespula*	67-69%
	Vespula	-	*Polistes*	30-55%
	Polistes	-	*Polistes*	76%
HYA	*Vespula*	-	*Dolichovespula*	80%
	Vespula	-	*Polistes*	73%

rabbit antisera and occasionally with human IgE (14, 17). Molecular data indicate that high similarity exists only between venom hyaluronidases (~90% identity), whereas the identity between phospholipases and antigens 5 is only around 65%. Furthermore, in CCD-positive sera, a substantial part of the observed cross-reactivity may be due to carbohydrate. During allergy testing, up to 90% of patients with vespid venom allergy react with all Vespinae venoms, but usually most strongly with *Vespula* venom, which is consistent with primary sensitisation by *Vespula* stings in the majority of patients (2). *Vespula* venom is therefore considered appropriate for diagnosis and immunotherapy in the vast majority of patients.

The clinical relevance of cross-reactivity with *Dolichovespula* venom in European patients is largely unknown because stings by these less aggressive wasps are rare and discrimination between *Dolichovespula* and *Vespula* species in the field is unfeasible. In contrast, hornet (*Vespa crabro*) stings are well documented as a cause of anaphylaxis and even perhaps associated with particularly severe reactions (18). Reciprocal IgE inhibition in patients with a history of hornet sting allergy, revealed primary *Vespula* allergy in most cases (confirming the clinical relevance of cross-reactivity with hornet venom) but true sensitisation to *Vespa* venom may be present in a subset of patients (19, 20). While *Vespula* venom presumably is sensitive enough to identify most patients with genuine *Vespa* or *Dolichovespula* allergy, it might not provide full protection when used for venom immunotherapy.

Cross-reactivity between yellow jacket (*Vespinae*) and paper wasp (*Polistes, Polistinae*) venoms is limited, due to a sequence identity of less than 60% between *Vespinae*

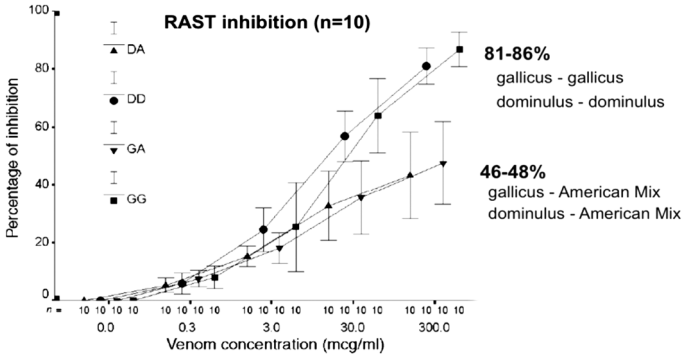


Fig. 1. IgE inhibition between European and American species of *Polistes.* Reproduced from Severino MG, Campi P, Macchia D et al. European Polistes venom allergy. Allergy 2006;61:860-863, with permission from Blackwell publishing Ltd.

and *Polistinae* phospholipases and antigens 5 (14, 21). Nevertheless, around 50% of *Vespula*-allergic patients still react to allergy tests with *Polistes* venom and vice versa (19, 22). Careful identification of the primary venom in *Vespula-Polistes* double-positive patients is of great importance in Mediterranean countries where stings by the thermophilic paper wasps can be more frequent than stings by the other Hymenoptera. Here the majority of patients with allergic reactions after a paper wasp sting may show primary sensitisation to *Polistes* venom while cross-reactivity with *Vespula* venom is less important (19-22). Due to incomplete cross-reactivity between European (*dominulus, gallicus*) and American *Polistes* species (*fuscatus, exclamans*) (Fig. 1), diagnosis and immunotherapy should be carried out with venoms from local species (22, 23).

Disclosure

The author states no conflicts of interest.

References

1. Egner W, Ward C, Brown DL, Ewan PW. The frequency and clinical significance of specific IgE to both wasp (Vespula) and honey-bee (Apis) venoms in the same patient. Clin Exp Allergy 1998;**28**:26-34.
2. Müller UR. Insect Sting Allergy. Clinical picture, diagnosis and treatment. Stuttgart, New York: Gustav Fischer; 1990.
3. Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy 2009;**64**:543-548.
4. Reisman RE, Wypych JI, Lazell MI. Further studies in patients with both honeybee- and yellow-jacket-venom-specific IgE. Int Arch Allergy Appl Immunol 1987;**82**:190-194.
5. Straumann F, Bucher C, Wüthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Int Arch Allergy Immunol 2000;**123**:268-274.
6. Hemmer W, Focke M, Kolarich D, Dalik I, Götz M, Jarisch R. Identification by immunoblot of venom glycoproteins displaying immunoglobulin E-binding N-glycans as cross-reactive allergens in honeybee and yellow jacket venom. Clin Exp Allergy 2004;**34**:460-9.
7. Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhrl S et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol 2001;**108**:1045-1052.
8. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hübsch-Müller C, Enk A. In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. Allergy 2006;**61**:1220-1229.
9. Jin C, Focke M, Léonard R, Jarisch R, Altmann F, Hemmer W. Re-assessing the role of hyaluronidase in yellow jacket venom allergy. J Allergy Clin Immunol 2010;**125**:184-90.
10. Seismann H, Blank S, Braren I, Greunke K, Cifuentes L, Grunwald T, Bredehorst R, Ollert M, Spillner E. Dissecting cross-reactivity in hymenoptera venom allergy by circumvention of alpha-1,3-core fucosylation. Mol Immunol 2010;**47**:799-808.
11. Hoffman DR, Jacobson RS. Allergens in Hymenoptera venom XXVII: Bumblebee venom allergy and allergens. J Allergy Clin Immunol 1996;**97**:812-821.
12. Hoffman DR. Allergens in Hymenoptera venoms XVI. Studies of the structures and cross-reactivities of vespid venom phospholipases. J Allergy Clin Immunol 1986;**78**:337-343.
13. Bucher C, Korner P, Wüthrich B. Allergy to bumblebee venom. Curr Opin Allergy Clin Immunol 2001;**1**:361-365.
14. Hoffman DR, El-Choufani SE, Smith MM, de Groot H. Occupational allergy to bumblebees: Allergens of Bombus terrestris. J Allergy Clin Immunol 2001;**108**:855-860.
15. King TP, Joslyn A, Kochoumian L. Antigenic cross-reactivity of venom proteins

from hornets, wasps and yellowjackets. J Allergy Clin Immunol 1985;**75**:621-628.
16. Golden DB, Breisch NL, Hamilton RG, Guralnick MW, Greene A, Craig TJ et al. Clinical and entomological factors influence the outcome of sting challenge studies. J Allergy Clin Immunol 2006;**117**:670-675.
17. King TP. Immunochemical studies of yellowjacket venom proteins. Mol Immunol 1983;**20**:297-308.
18. Antonicelli L, Bilò MB, Napoli G, Farabollini B, Bonifazi F. European hornet (Vespa crabro) sting: a new risk factor for life-threatening reaction in hymenoptera allergic patients? Eur Ann Allergy Clin Immunol 2003;**35**:199-203.
19. Blanca M, Garcia F, Miranda A, Carmona MJ, Garcia J, Fernandez J et al. Determination of IgE antibodies to Polistes dominulus, Vespula germanica and Vespa crabro in sera of patients allergic to vespids. Allergy 1991;**46**:109-114.
20. Kosnik M, Korosec P, Silar M, Music E, Erzen R. Wasp venom is appropriate for immunotherapy of patients with allergic reactions to the European hornet sting. Croat Med J 2002;**43**:25-27.
21. Caruso B, Bonadonna P, Severino MG, Manfredi M, Dama A, Schiappoli M et al. Evaluation of the IgE cross-reactions among vespid venoms. A possible approach for the choice of immunotherapy. Allergy 2007;**62**:561-564.
22. Severino MG, Campi P, Macchia D, Manfredi M, Turillazzi S, Spadolini I et al. European Polistes venom allergy. Allergy 2006;**61**:860-863.
23. Sanchez F, Blanca M, Fernandez J, Miranda A, Terrados A, Torres MJ et al. Comparative study between European and American species of Polistes using sera from European sensitized subjects. Clin Exp Allergy 1995;**25**:281-287.

FUTURE STRATEGIES IN DIAGNOSIS OF INSECT VENOM ALLERGY

Adriano Mari

Center for Clinical and Experimental Allergology, IDI-IRCCS, Rome, Italy
and Allergy Data Laboratories sc, Latina, Italy

Diagnosis of Hymenoptera venom allergy has evolved dramatically since the very early days when tests were based on the use of whole-body extracts (1, 2). Whole-body extracts of wasp and bee were in use up until the late nineteen seventies (3), and are still in use for ant venom allergy testing and immunotherapy (4). The direct collection of venom material from venom sacs has reduced the amount of unnecessary peptides, not related to allergic sensitisation, in Hymenoptera venom preparations. However, allergenic preparations from pollen, epithelia, fruits, and seeds still require use of whole tissue. The number of diagnostic tools for allergy has multiplied in the last 20 years, which has led not only to a better clinical understanding of allergies, but also to a dramatic increase in the number of newly identified allergens (Fig. 1). To date, the Allergome database (www.allergome.org) lists 1676 allergenic molecules, excluding isoforms and epitopes, and 1169 isoforms of allergenic molecules (5).

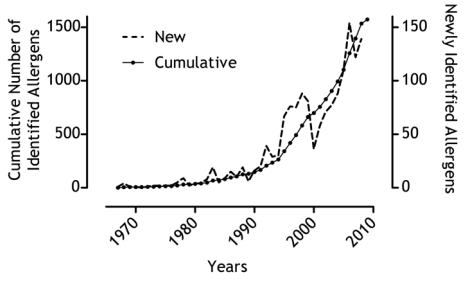


Fig. 1. Increase in the number of identified allergens.

Currently, 54 species of Hymenoptera worldwide have been described as allergenic (Table 1) (6). Compared to many other sources of allergens, identification of the allergenic components of Hymenoptera venom started very early (7, 8-11). This was partly helped by the interest of scientists who were outside the field of immunology research, and soon led to a number of Hymenoptera antigens becoming commercially available (12, 13). The identification and characterisation of allergenic molecules in bee, wasp, and ant venom continues and more than 160 articles have been published on the topic so far (5). Biochemical, immunochemical, molecular biology, and bioinformatics tools have been applied to characterise the allergenic molecules of Hymenoptera venom. Currently, 90 allergenic molecules with 73 isoforms have been characterised from 34 species, and the allergenic components of a further 22 species of Hymenoptera await characterisation. Allergenic molecules are grouped depending on their biochemical function and structure and, in general, official WHO-IUIS nomenclature reflects this grouping, unless numerous exceptions exist (14). The knowledge of which molecules in venoms are antigenic is the basis for improving the diagnosis of allergies in general, and more specifically,

Table 1. Allergenic bees, wasps, and ants. Species marked with * have at least one allergenic molecule being characterised. Data retrieved on August 10, 2009 from www.allergome.org (5).

Apis cerana *	*Myrmecia pilosula* *	*Polybia paulista* *
Apis dorsata *	*Myrmecia pyriformis*	*Solenopsis aurea*
Apis florea *	*Myrmecia simillima*	*Solenopsis geminata* *
Apis mellifera *	*Myrmecia tarsata*	*Solenopsis invicta* *
Apis mellifera scutellata	*Odontomachus bauri*	*Solenopsis richteri* *
Bombus pennsylvanicus *	*Pachycondyla chinensis* *	*Solenopsis saevissima* *
Bombus terrestris *	*Pachycondyla sennaarensis*	*Solenopsis xyloni*
Brachyponera chinensis	*Pachycondyla solitaria*	*Tetraponera rufonigra*
Camponotus pennsylvanicus	*Pogonomyrmex rugosus*	*Vespa crabro* *
Dolichovespula arenaria *	*Polistes annularis*	*Vespa magnifica* *
Dolichovespula maculata *	*Polistes apachus*	*Vespa mandarinia* *
Monomorium pharaonis	*Polistes dominulus* *	*Vespa velutina* *
Myrmecia forficata	*Polistes exclamans* *	*Vespula germanica* *
Myrmecia gratiosa	*Polistes fuscatus* *	*Vespula maculifrons* *
Myrmecia gulosa	*Polistes gallicus* *	*Vespula pensylvanica* *
Myrmecia ludlowi	*Polistes hebraeus* *	*Vespula squamosa* *
Myrmecia nigriscapa	*Polistes metricus* *	*Vespula vidua* *
Myrmecia nigrocincta	*Polistes versicolor*	*Vespula vulgaris* *

Hymenoptera venom sensitisation. In the last ten years an increasing number of publications have been published on molecule-based allergy diagnosis. The first allergenic molecules to become commercially available were the pollen allergens, and allergenic molecules are now available for most allergenic sources. It is now possible to obtain a profile of a patient's IgE using microarray technology to test a combination of molecular preparations (15).

Profiling the patient's IgE sensitisation also enables the evaluation of structurally homologous molecules (16, 17). Although this is generally the case for a group of allergens in IgE-mediated disease, structural homology does not necessarily mean IgE co-recognition. IgE co-recognition must be explored in individual patients and it is feasible that a combination of the technological approaches listed above can provide answers to long-standing problems, such as double-positive patients (18). The use of cloned allergens deposited in minute amounts on biochips will reduce the need for venom raw material and reduce the costs associated with testing. Moreover, several classes of immunoglobulins are of interest in the immune response to Hymenoptera venom and microarray technology-based diagnosis will enable simultaneous measurement of IgE, IgG4, and total IgG. This approach will lead to a better understanding of the patient's status before and after immunotherapy. A robust diagnostic strategy based on these innovative tools should be the starting point for future molecule-based immunotherapy.

Disclosure

The author states no conflicts of interest.

References

1. Langlois C, Shulman S, Kozmycz S, Arbesman CE. The allergic response to stinging insects. VI. Fractionation of whole body venom sac extracts of wasp. J Allergy 1966;**37**:359-365.
2. Shulman S, Langlois C, Miller JB, Arbesman CE. The allergic response to stinging insects. V. Fractionation of whole body and venom sac extracts of bee. J Allergy 1966;**37**:350-358.
3. Hunt KJ, Sobotka AK, Valentine MD, Yunginger JW, Lichtenstein LM. Sensitization following Hymenoptera whole body extract therapy. J Allergy Clin Immunol 1978;**61**:48-53.
4. Judd CA, Parker AL, Meier EWA, Tankersley MS. Successful administration of a 1-day imported fire ant rush immunotherapy protocol. Ann Allergy Asthma Immunol 2008;**101**:311-315.
5. http://www.allergome.org
6. Mari A, Rasi C, Palazzo P, Scala E. Allergen databases: current status and perspectives. Curr Allergy Asthma Rep 2009;**9**:376-383.
7. Mackler BF, Russell AS, Kreil G. Allergenic and biological activities of melittin from honey bee venom. Clin Allergy 1972;**2**:317-323.
8. King TP, Sobotka AK, Kochoumian L, Lichtenstein LM. Allergens of honey bee venom. Arch Biochem Biophys 1976;**172**:661-671.
9. Hoffman DR, Shipman WH. Allergens in bee venom. I. Separation and identification of the major allergens. J Allergy Clin Immunol 1976;**58**:551-562.
10. Hoffman DR. Allergens in bee venom. III. Identification of allergen B of bee venom as an acid phosphatase. J Allergy Clin Immunol 1977;**59**:364-366.
11. King TP, Sobotka AK, Alagon A, Kochoumian L, Lichtenstein LM. Protein allergens of white-faced hornet, yellow hornet, and yellow jacket venoms. Biochemistry 1978;**17**:5165-5174.
12. Habermann E. Bee and wasp venoms. Science 1972;**177**:314-322.
13. Sobotka AK, Franklin RM, Adkinson NF, Jr., Valentine M, Baer H, Lichtenstein LM. Allergy to insect stings. II. Phospholipase A: the major allergen in honeybee venom. J Allergy Clin Immunol 1976;**57**:29-40.
14. Chapman MD. Allergen nomenclature. Clin Allergy Immunol 2008;**21**:47-58.
15. Harwanegg C, Hiller R. Protein microarrays for the diagnosis of allergic diseases: state-of-the-art and future development. Clin Chem Lab Med 2005;**43**:1321-1326.
16. Krause S, Reese G, Randow S, Zennaro D, Quaratino D, Palazzo P et al. Lipid transfer protein (Ara h 9) as a new peanut allergen relevant for a Mediterranean allergic population. J Allergy Clin Immunol 2009;**124**:771-778.
17. Gadermaier G, Harrer A, Girbl T, Palazzo P, Himly M, Vogel L et al. Isoform identification and characterization of Art v 3, the lipid-transfer protein of mugwort pollen. Mol Immunol 2009;**46**:1919-1924.
18. Muller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy 2009;**64**:543-548.

INSECT STINGS AND BITES - NOT ONLY HYMENOPTERA

Peter Schmid-Grendelmeier

Allergy Unit Dept of Dermatology, Zürich, Switzerland

Allergic reactions to insects other than hymenoptera are not commonly reported. However, the stings or bites of mosquitoes, or direct contact with caterpillars, and silk worms can be responsible for large local reactions (LLRs) and severe anaphylactic reactions. Other arthropods, such red mosquito larvae, cockroaches, ladybugs or silverfish may play an important role as inhalant allergens in causing asthma. Due to the fact that diagnostic tools are not yet well standardised, their role as allergens may well be underestimated. The use of a single or recombinant allergen could improve the diagnosis and possibly also therapeutic management of such allergies.

Introduction

Insects belong to the phylum of the arthropods - the largest animal family on earth including:

- *Arachnoidea* (spiders, ticks, scorpions and mites)
- Centipedes and millipedes
- *Crustaceae* (Shrimps, Crabs, Lobster, Crawfish, Woodlice)
- Insects

Allergic reactions to all of these orders have been reported. Allergies to various species of mites (house dust mites, storage mites) are well known, and allergic reactions to some ticks such as the European pigeon tick (*Argas reflexus*) have also been well characterised (1). Bites, stings or local contact with arthropods do not only result in allergic reactions (see Table 1), but local pain and large reactions can also be due to toxic substances; some substances may induce systemic toxic or even lethal reactions. However, possibly the most important risk to public health is the transmission of vector-borne diseases - such as malaria or onchocercosis.

Insects as allergens

The insect class is estimated to include more than 6 million species - of which only about one million are currently known. Allergic reactions to Hymenoptera are well known. Besides honeybees and various wasp species, imported red fire ants are a common cause of local and systemic allergic reactions. Their venom is injected by bites not stings and contains similar alkaloids to wasp venom. Cross-reactions with different Hymenoptera venom can occur. Allergen-specific immunotherapy is possible but is still mostly based on whole-body extracts, with limited success (2). Due to the increasing number of imported goods, the red fire ant has been brought to Europe where it has been responsible for anaphylactic reactions (3). Also, rare cases of anaphylaxis due to other ants, such as the red harvest ant, have been observed (4).

Allergic reactions to mosquitoes

Mosquitoes occur worldwide and allergic reactions, including severe local and systemic reactions to mosquito bites, may involve immunoglobulin E, immunoglobulin G, and T-lymphocyte-mediated hypersensitivities in response to allergens in mosquito saliva. Naturally acquired desensitisation to mosquito saliva may occur during childhood or during long-term exposure to mosquitoes. As the availability of mosquito salivary preparations for use in skin tests and *in vitro* tests is very limited, allergic reactions to mosquito bites are likely under-diagnosed and under-treated.

Typical local cutaneous reactions to mosquito bites consist of immediate wheals and flares, peaking 20 minutes after the bite, and delayed pruritic indurated papules peaking at 24-36 h, then diminishing over several days or weeks (5). LLRs to mosquito bites consist of itchy, red, warm swellings appearing within minutes, and itchy papules, ecchymotic, vesiculated, blistering, bullous reactions or even arthus reactions, appearing 2-6 h after the bites and persisting for days or weeks. Only reactions with large or atypical (ecchymotic or vesiculated) local reactions or systemic reactions should be described as 'mosquito allergy'. 'Skeeter syndrome' is defined as a mosquito saliva-induced large local inflammatory reaction that is sometimes accompanied by low-grade fever and often misdiagnosed as cellulitis. Systemic reactions following mosquito bites include angioedema, generalised urticaria, nausea, vomiting, wheezing and other manifestations of anaphylaxis. Individuals at increased risk of severe reactions to mosquito bites include those with a high level of exposure, for example, outdoor workers. In addition, severe reactions can be observed in individuals with weak natural immunity, such as infants and young children or those who have had no previous exposure to indigenous mosquitoes, for example, immigrants or visitors. Individuals with Epstein-Barr virus-associated natural killer/T lymphoproliferative diseases may even develop necrotic skin ulcers at the sites of mosquito bites as these play an important role in the pathogenesis of mosquito allergy. Cytotoxic chemotherapy successfully improves the symptoms of mosquito allergy in these patients.

Non-IgE-mediated mast cell degranulation may also be involved in the mechanism of mosquito-bite induced allergic responses. Recently, mosquito bite-induced skin mast cell degranulation leading to fluid extravasation and recruitment of neutrophils at the site of the mosquito bite was reported in a murine model. *Anopheles stephensi* saliva directly induces isolated connective tissue-type but not mucosal-type mast cells to degranulate *in vitro* in the absence of IgE antibodies (6). This effect may explain the observation that patients with mastocytosis often react very strongly to mosquito bites.

Skin prick tests to mosquito saliva are commercially available; however, their sensitivity and specificity is limited. Mosquito saliva can be obtained only by dissecting out the salivary glands or by direct collection of saliva from living female mosquitoes, which is very time consuming and thus is not practical. Due to the lack of salivary preparations, allergic reactions to mosquito bites are under-diagnosed and under-treated. Recent diagnostic approaches utilising recombinant mosquito allergens are more promising. Salivary proteins of *Ae. aegypti*, including rAed a 1, rAed a 2, rAed a 3, and rAed a 4 have been characterised (7-9).

Based on clinical and experimental observations, the natural history of sensitisation and subsequent desensitisation to the saliva injected by mosquito bites has been documented (10). Natural desensitisation may take years to occur, or might never occur, as mosquito-allergic individuals usually try to avoid getting bitten by mosquitoes. Natural desensitisation probably occurs during childhood and adolescence, and thus infants and young children may be at increased risk of having severe reactions to mosquito bites. Based on the these observations, immunotherapy with injections of gradually increasing doses of mosquito saliva proteins may be expected to prevent reactions to mosquito bites, and immunotherapy using injections of mosquito whole-body extracts have confirmed this (10, 11). A recent study

using whole-body extracts of single insects (cockroach, housefly, or mosquito) and a mix of these allergens for immunotherapy showed no significant difference between mixed and single group patients for changes in clinical and immunological parameters. A positive correlation was observed between an increase in IgG4 and clinical improvement (12). Immunotherapy to mosquito deserves further consideration, since mosquito allergens contained in dust have been proposed to induce symptoms of allergic asthma (13).
Immunotherapy with mosquito allergens is, however, not widely used in mosquito allergy, because currently commercially available mosquito whole-body extracts contain low amounts of mosquito saliva proteins and many non-salivary proteins - which are ineffective in down-regulating the specific immune responses to mosquito salivary allergens and may even cause additional sensitisation. Possibly the use of single native or recombinant mosquito allergens might significantly add to improved IT and achieve results comparable to honeybee or wasp hypersensitivity.

Allergic reactions to chironomid larvae (Red Mosquito/midge larvae)
Chironomids are insects that inhabit wetlands. In countries such as Sudan, Egypt, but also The United States and Japan they are frequently the cause of serious allergies. In Europe, chironomid allergy is rare and has only been described in patients who handle chironomid larvae contained in certain fish foods. Thus, it is mostly known as "fishfood" allergy - manifesting mostly with rhinoconjuncitivits or even asthmatic symptoms while feeding fish. Both skin prick tests and kits to detect, specific serum IgE are commercially available for diagnosis. *In vitro* techniques have demonstrated cross-reactivity with common mosquitoes (14).

Allergic reactions to ladybugs
Recently, reports of allergic reactions to larvae of ladybugs (*Harmonia axyridisi*) have been published (15). These larvae cause rhinoconjunictivits or allergic asthma predominantly during the winter months. Sensitisation can be detected by skin tests or specific IgE in the serum. Analysis of proteins has identified two specific allergens, Har a 1 and Har a 2, and *in vitro* cross-reactivity with *Blattella germanica* has been observed. Preliminary data suggest that the 10 kD protein Har a 1 is specific for ladybugs and may be suitable for measuring IgG antibodies and for assessing environmental exposure. As treatment approaches are limited, different avoidance techniques have been tried, including chemicals, traps, sound waves, and painting the house a dark colour to discourage swarming. Although none of these methods were consistently successful, the best results were reported when treating the outside of the house with pyrethroids before cold weather. Whether the allergens are primarily derived from the brown liquid ladybugs excrete, making distinctive marks on the walls, or from the debris of dead beetles is not clear. Immunotherapy with extracts of *H. axyridis* has been reported anecdotally, but to date, no controlled trials have been performed. As controlled trials are essential, it is unlikely that commercial extracts will be approved for immunotherapy for several years.

Allergic reactions to cockroaches
Cockroaches are frequently found in homes worldwide and are well adapted to modern human residential areas. Over 3,500 cockroach species exist worldwide and about 50 species live in or around human houses. Excreta, regurgitated food, excreted body fluid, dead bodies, and castovers contain allergens and elicit strong allergic symptoms in sensitised individuals. Cockroaches are an important inhalant allergen in many areas of the world. In some regions such as Brazil they are even the most common cause of allergic asthma (16). Major IgE binding components of cockroach were found to be concentrated in the faeces.
Exposure to cockroach allergens in the first three months of life has been associated with repeated wheezing and asthma. The principal domestic cockroach species are *Blattella germanica* and *Periplaneta americana*. Both species produce several potent allergens,

Table 1. Insects other than Hymenoptera with reported allergic symptoms

Family	Local reactions	Asthma	Anaphylaxis
Mosquitoes	+++	++	+
Red midge larvae	+	++	
Cockroaches		+++	+
Silverfish		++	
Silkworm	+	++	
Caterpillar	+++	+	
Ladybugs		+++	

including Bla g 2 (inactive aspartic proteinase), Bla g 4 (calycin), Bla g 5 (glutathione-S-transferase), the group 1 cross-reactive allergens Bla g 1 and Per a 1, and tropomyosin. The 3-dimensional structures of several cockroach allergens are known, and biologically active recombinant allergens have been produced in high-level expression vectors. The use of recombinant cockroach allergens should facilitate investigation of the mechanisms of cockroach-induced asthma and may lead to the development of new approaches to asthma treatment. Environmental allergen measurements of Bla g 1 and Bla g 2 have allowed exposure levels that cause allergic sensitisation to be established. Abatement studies have shown that a sustained decrease in cockroach allergen levels is difficult but can be accomplished by professional application of insecticides, together with rigorous household cleaning. Cockroach asthma is an important public health problem that affects patients who are the least likely to be compliant with treatment with asthma medications or environmental control and may also play an important role in inner-city asthma morbidity. Patient education, improvements in housing and environmental and immunologic treatment strategies are likely to be the most successful approaches to reduce the prevalence of cockroach-induced asthma (18, 19).

Allergic reactions to other insects

Silverfish

Silverfish (*Lepisma saccharina*) are the most primitive living insect. Significant levels of silverfish allergens can be present in house dust, even in houses where the inhabitants are unaware of their presence. Although inhalant allergies to silverfish have been described (19), there are currently no silverfish extract for diagnosis of allergic diseases commercially available. Identification of optimal extraction conditions and characterisation of allergenic extracts are the first steps to obtain an effective allergen preparation suitable for diagnosis and therapy, and will be useful as a reference for assessing silverfish allergen exposure in different indoor environments. A silverfish tropomyosin, Lep s 1, has been cloned and characterised and is the first allergen identified in silverfish extract. rLep s 1 displayed biological activity, suggesting that it could be regarded as a useful tool to study the role of silverfish tropomyosin in the sensitisation to invertebrate allergic sources.

Caterpillars

Caterpillars are wormlike, larval forms of butterflies and moths of the insect order Lepidoptera. Most caterpillars pose little threat to humans and caterpillars from only 12 families throughout the world are capable of inflicting serious human injuries. Exposure to processionary tree caterpillars can, however, cause an allergic reaction in humans. Contact urticaria and contact dermatitis are the most common manifestations. Systemic reactions

due to inhalation/ingestion of the caterpillar's airborne toxin-containing hair and spines occur less frequently and have been reported in forestry workers (20, 21).

Silk worm

Silk worms (*Bombyx mori*) can induce allergic reactions, these result mostly from occupational exposure among silk farmers and workers in silk filatures. Many workers who are exposed to silkworm allergens develop asthma. Non-occupational exposure to silk can also result in sensitisation, but this occurs rarely (22). Allergic reactions can also be caused by inhaling or touching allergens from silk products such as quilts and clothes or eating pupa-containing foods (23, 24) Recently, arginine kinase has been identified as a major silkworm allergen (25).

Cross-reactivity between invertebrate allergens

Tropomyosin from a number of insects, such as the red mosquito larvae, has been described (26). As with other allergenic sources this raises the possibility that primary sensitisation against insect tropomyosins from inhalation, stings or bites could lead to a secondary food allergy. Thus, structural homology between tropomyosins from cockroaches, mites, and shrimp may explain clinical cases of the oral allergy syndrome or even severe anaphylaxis due to food allergy also called the "mite-cockroach-crustacean-syndrome". Recent reports have also shown cross-reactivity with tropomyosin from *ascaris lumbricoides.* Interestingly, infection with *A. lumbricoides* has been associated with lack of protection or even increased risk for allergen sensitisation and asthma symptoms in some studies, whereas infections with Schistosoma and hookworm were shown to promote protection (27, 28). All these allergens, possibly due to their evolutionary distance from human homologues, might be relevant for important food or inhalant allergies. The same could be relevant not only for cockroaches but also mosquitoes, ladybugs or silkworms (29), see Fig. 1.

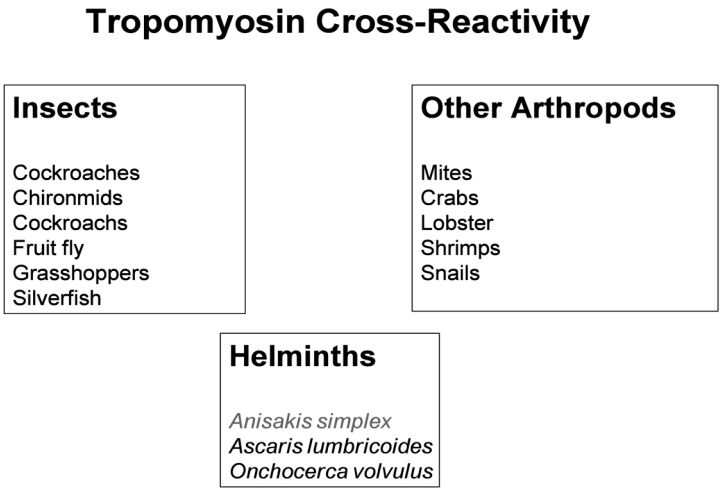


Fig. 1. Known cross-reactivity between various species and genera of insects, other arthropods and helminths

Other potential allergenic risks of insect bites and stings

Recently, a possible relationship between tick bites and meat allergy due to antibodies against mammalian carbohydrates such as galactose-alpha-1,3-galactose has been raised. Based on current evidence, it might be possible that tick bites can give rise to IgE responses against both carbohydrates and tick-derived proteins. Glycosylation of proteins might create a risk for severe hypersensitivity against these otherwise non-allergenic substances and may explain why some recombinant molecules as monoclonal antibodies can provoke severe anaphylactic reactions when glycosylated (30-32).

Conclusions

In conclusion, allergic reactions occur to various insects besides Hymenoptera. These include not only local reactions but in rare cases also severe anaphylactic reactions. Inhalation of insect allergens or possibly also their presence in food might be important causes of asthma. Cross-reactivity with potent allergens such as tropomyosin from invertebrates such as mites or some helminthic parasites occurs. However, mainly due to the limited accuracy of the available diagnostic tools the significance of these allergies is still unknown. The use of recombinant allergens and new technologies such as microarray-based allergy diagnosis may further contribute to a better understanding of allergies to insects other than Hymenoptera.

Disclosure

The author states no conflicts of interest.

References

1. Kleine-Tebbe J, Heinatz A, Gräser I, Dautel H, Hansen GN, Kespohl S, et al. Bites of the European pigeon tick (Argas reflexus): Risk of IgE-mediated sensitizations and anaphylactic reactions J Allergy Clin Immunol 2006;**117**:190-195.
2. Solley GO, Vanderwoude C, Knight GK. Anaphylaxis due to Red Imported Fire Ant sting. Med J Aust 2002;**176**:521-523.
3. Fernández-Meléndez S, Miranda A, García-González JJ, Barber D, Lombardero M. Anaphylaxis caused by imported red fire ant stings in Málaga, Spain. J Investig Allergol Clin Immunol 2007;**17**:48-49.
4. Seebach JD, Bucher C, Anliker M, Schmid-Grendelmeier P, Wüthrich B. Ant venoms: a rare cause of allergic reactions in Switzerland. Schweiz Med Wochenschr 2000;**130**:1805-1813.
5. Peng Z, Simons FE. Advances in mosquito allergy. Curr Opin Allergy Clin Immunol 2007;**7**:350-354.
6. Depinay N, Hacini F, Beghdadi W, Peronet R, Mécheri S. Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites. J Immunol 2006;**176**:4141-4146.
7. Wongkamchai S, Vaiyavatjamai P, Wanachiwanawin D, Wisuthsarewong W, Kulthanan K, Eakpo P. A dot-blot and immuno-blot assay for the detection of mosquito saliva specific IgE in mosquito bite allergic subjects. J Dermatol Sci 2009;**53**:155-157.
8. Peng Z, Xu W, Lam H, Cheng L, James AA, Simons FE. A new recombinant mosquito salivary allergen, rAed a 2: allergenicity, clinical relevance, and cross-reactivity. Allergy 2006;**61**:485-490.
9. Wang Q, Beckett A, Simons FER, Peng Z. Mosquito saliva-capture ELISA is more sensitive and specific than the UniCAP test in the diagnosis of mosquito allergy. Ann Allergy Asthma Immunol 2007;**99**:199-200.
10. Peng Z, Simons FER. Mosquito allergy: immune mechanisms and recombinant salivary allergens. Int Arch Allergy Immunol 2004;**133**:198-209.
11. Ariano R, Panzani RC. Efficacy and safety of specific immunotherapy to mosquito bites. Allerg Immunol (Paris) 2004;**36**:131-138.
12. Srivastava D, Singh BP, Arora N, Gaur SN. Clinico immunological study on immunotherapy with mixed and single insect illergens. J Clin Immunol 2009;**29**:665-673.
13. Kausar MA, Vijayan VK, Bansal SK, Menon BK, Vermani M, Agarwal MK. Mosquitoes as sources of inhalant allergens: clinicoimmunologic and biochemical

studies. J Allergy Clin Immunol 2007;**120**:1219-1221.
14. Cabrerizo Ballesteros S, de Barrio M, Baeza ML, Rubio Sotés M. Allergy to chironomid larvae (red midge larvae) in non professional handlers of fish food. J Investig Allergol Clin Immunol 2006;**16**:63-68.
15. Nakazawa T, Satinover SM, Naccara L, Goddard L, Dragulev BP, Peters E et al. Asian ladybugs (Harmonia axyridis): a new seasonal indoor allergen. J Allergy Clin Immunol 2007;**119**:421-427.
16. Arruda LK, Vailes LD, Ferriani VP, Santos AB, Pomés A, Chapman MD. Cockroach allergens and asthma. J Allergy Clin Immunol 2001;**107**:419-428.
17. Wang J, Visness C, Calatroni A, Gergen P, Mitchell H, Sampson H. Effect of environmental allergen sensitization on asthma morbidity in inner-city asthmatic children. Clin Exp Allergy 2009;**39**:1381-1389.
18. Perzanowski MS, Platts-Mills TA. Further confirmation of the relevance of cockroach and dust mite sensitization to inner-city asthma morbidity. Clin Exp Allergy 2009;**39**:1291-1293.
19. Barletta B, Di Felice G, Pini C Biochemical and molecular biological aspects of silverfish allergens. Protein Pept Lett 2007;**14**:970-974.
20. Vega JM, Moneo I, Armentia A, Fernández A, Vega J, De La Fuente R et al. Allergy to the pine processionary caterpillar (Thaumetopoea pityocampa). Clin Exp Allergy 1999;**29**:1418-1423.
21. Shkalim V, Herscovici Z, Amir J, Levy Y. Systemic allergic reaction to tree processionary caterpillar in children. Pediatr Emerg Care 2008;**24**:233-235.
22. Wen CM, Ye ST, Zhou LX, Yu Y. Silk-induced asthma in children: a report of 64 cases. Ann Allergy 1990;**65**:375-378.
23. Ji KM, Zhan ZK, Chen JJ, Liu ZG: Anaphylactic shock caused by silkworm pupa consumption in China. Allergy 2008;**63**:1407-1408.
24. Borelli S, Stern A, Wüthrich B. A silk cardigan inducing asthma. Allergy 1999;**54**:900-901.
25. Liu Z, Xia L, Wu Y, Xia Q, Chen J, Roux KH. Identification and characterization of an arginine kinase as a major allergen from silkworm (Bombyx mori) larvae. Int Arch Allergy Immunol 2009;**150**:8-14.
26. Jeong KY, Hong CS, Yong TS. Allergenic tropomyosins and their cross-reactivities Protein Pept Lett. 2006;**13**:835-845.
27. Santos AB, Rocha GM, Oliver C, Ferriani VP, Lima RC, Palma MS et al. Cross-reactive IgE antibody responses to tropomyosins from Ascaris lumbricoides and cockroach. J Allergy Clin Immunol. 2008;**121**:1040-1046.
28. Acevedo N, Sánchez J, Erler A, Mercado D, Briza P, Kennedy M et al. IgE cross-reactivity between Ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 2009;**64**:1635-1643.
29. Jenkins JA, Breiteneder H, Mills EN. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. J Allergy Clin Immunol 2007;**120**:1399-1405.
30. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med 2008;**358**:1109-1117.
31. Van Nunen SA, O'Connor KS, Clarke LR, Boyle RX, Fernando SL. An association between tick bite reactions and red meat allergy in humans. Med J Aust 2009;**190**:510-511.
32. Commins SP, Platts-Mills TA. Anaphylaxis syndromes related to a new mammalian cross-reactive carbohydrate determinant. J Allergy Clin Immunol 2009;**124**:652-657.

PART TWO

Treatment with venom immunotherapy

VENOM IMMUNOTHERAPY: INDICATION AND DURATION

Holger Mosbech

Allergy Unit, Copenhagen University Hospital, Denmark

Venom immunotherapy (VIT) is an effective treatment for reducing the risk of systemic and local reactions in individuals with Hymenoptera venom allergy (1, 2). The indication for VIT in hymenoptera venom allergic individuals is based on the outcome of diagnostic testing (skin tests and specific IgE) and the presence of other risk factors for a future severe reaction (2). However, some groups of patients, such as beekeepers, pregnant women, and patients on antihypertensive treatment with beta-blockers or ACE-inhibitors present special circumstances. Beekeepers who continue to keep bees have a high likelihood of a future sting and possible multiple stings. The most effective way to reduce this likelihood is to give up bee keeping, however, many patients are reluctant to do this. Alternative approaches in case of treatment failures could involve giving a double dose of VIT with a sting challenge to test effectiveness and/or the issue of two EpiPens®. In pregnant women, the start of VIT should be postponed until after the birth, but if already on maintenance dose, a VIT without complications could be continued. Patients receiving treatment with beta-blockers should consider changing to a cardioselective version, or to a drug from another group if otherwise equivalent. ACE-inhibitor treatment has likewise been suspected of deteriorating allergic reaction although there is no strong evidence at present.

Clinical practice guidelines for starting and stopping VIT in Europe and US are rather similar (2, 3). The duration of VIT should clearly be determined by the duration of the protective effect that it offers. A review of clinical studies reported that 7.5-19% of patients have a systemic reaction when re-stung 3 and 7 years after discontinuation of VIT (2). Often, the systemic reactions following a re-sting are less severe than the original reaction (4). The actual reported efficacy rates varies considerably in different studies depending on type of insect, sting challenge vs. field sting, age group, classification of symptoms, duration of treatment, patient selection etc. A risk factor should be identified within one study group, ideally with the help of multivariate analyses. No such study exists, however, in general, adults are more likely to suffer a relapse after stopping VIT than children and are also more likely to suffer side effects. Patients with honeybee venom allergy, severe reactions to VIT, and patients who underwent VIT for three years versus those who were treated for five years or more are also more likely to suffer a relapse (5). Elevated basal serum tryptase levels (6, 7), mastocytosis, and elevated skin sensitivity at the time of ending VIT are also indicative of a higher risk of relapse (2). In patients who received five or more years of VIT the proportion who experienced systemic reactions to a resting did not increase from three to nine years after stopping treatment (5). Some studies have recommended a negative sIgE test as stopping criteria (8), however this does not seem valid (9).

In conclusion, the 2005 EAACI guidelines (2) are still valid. A treatment duration of five years or lifelong, depending on risk factors, is recommended, and the duration of protection after treatment is likely to be >10 yrs or lifelong, depending on the individual patient's risk factors.

Disclosure
The author states no conflicts of interest.

References

1. Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Outcomes of allergy to insect stings in children, with and without venom immunotherapy. N Engl J Med 2004;**351**:668-674.
2. Bonifazi F, Jutel M, Bilo BM, Birnbaum J, Muller U, EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005:**60**;1459-1470.
3. Moffitt JE, Golden DB, Reisman RE, Lee R, Nicklas R, Freeman T et al. Stinging insect hypersensitivity: a practice parameter update. J Allergy Clin Immunol 2004;**114**:869-886.
4. Lerch E, Müller UR: Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. J Allergy Clin Immunol 1998;**101**:606-12
5. Golden DB, Kagey-Sobotka A, Lichtenstein LM. Survey of patients after discontinuing venom immunotherapy. J Allergy Clin Immunol 2000:**105**;385-390.
6. Haeberli G, Brönnimann M, Hunziker T, Müller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003;**33**:1216-1220.
7. Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase. J
8. Allergy Clin Immunol 2009;**124**:1047-54. Golden DB. Discontinuing venom immunotherapy. Curr Opin Allergy Clin Immunol 2001;**1**:353-356.
9. Reisman RE, Lantner. Further observations of stopping venom immunotherapy: comparison of patients stopped because of a fall in serum venom-specific IgE to insignificant levels with patients stopped prematurely by self-choice. J Allergy Clin Immunol 1989;**83**:1049-1054.

HOW TO REDUCE SIDE EFFECTS DURING VENOM IMMUNOTHERAPY?

M. Beatrice Bilò and Floriano Bonifazi

Allergy Unit, Department of Internal Medicine, Allergy, Immunology and Respiratory Diseases, University Hospital, Ancona, Italy

Introduction

There is no doubt that venom immunotherapy (VIT) is a highly effective treatment, which lessens the risk of a subsequent systemic reaction (SR), reduces morbidity and mortality, and improves the patients' health-related quality of life (1, 2). However, local and systemic reactions, especially during the initial phase of VIT, are not rare (1-3). Large local reactions (LLRs) to injections are common, and occur in up to 50% of patients. Although LLRs during VIT can be bothersome, they are not a risk factor for SR and do not usually necessitate a reduction in venom dose or prevent the physician from reaching full dose while up-dosing. The biggest risk of VIT is an anaphylactic reaction, and this represents a significant problem in the treatment of venom allergic patients. Other side effects of VIT, such as serum sickness, are extremely rare (3). Reports in the literature reveal a large variation (0-46%) in the incidence of side effects attributable to VIT (1, 3) (Table 1). However, comparison of the incidence of SR between different VIT protocols is problematic, as different investigators employ different classification systems for measuring the severity of adverse reactions (1). The study "Hymenoptera Venom Study III", on the safety of VIT revealed that 12% of patients that received VIT experienced SRs (327 in 1410 subjects); 9% of which were severe, 32% moderate, and 59% mild (4).

In a multicenter European prospective study evaluating data from 840 patients, side effects to VIT occurred in 20% of patients; the majority of these reactions were mild in severity, and only one third required medical treatment (5). An analysis of eight studies that enrolled at least 100 patients, reported that 20.3% of patients treated with VIT experienced side effects; SRs were reported in 2-16% of patients and adrenaline was administered to 0.2-5% of patients who experience a SR (6). However, according to some studies side effects of VIT are less frequent than those occurring during subcutaneous immunotherapy for inhalant allergens (7); in a prospective study comparing the frequency of side effects with VIT to aeroallergens, VIT with wasp venom had the highest frequency of patients reaching the maintenance dose without adverse reactions and the lowest frequency of side effects (8).

Risk factors of side effects

SRs during VIT pose a serious problem and presently there is no reliable test able to predict the risk of an SR. The finding that increased basophil sensitivity to allergen-specific *in vitro* stimulation is significantly associated with major side effects of VIT needs to be confirmed by further studies (9). To reduce the incidence of SR during VIT, it would be useful to define the risk factors. In a European multicenter study, a

Table 1. Venom immunotherapy and side effects

- Lockey RF et al. (4):
  12% of 1410 VIT treated subjects experienced 327 SRs
- Mosbech H et al. (5):
  20% of 840 patients had side effects
- Niedoszytko M et al. (6):
  20.3% of 3006 patients had side effects, among which SRs occurred in 2-16%*

Side effects were more frequent with honeybee venom extracts and during the incremental phase

SR: systemic reaction
VIT: venom-specific immunotherapy
* This review included reference (5).

greater risk of SRs was demonstrated during the incremental phase of VIT, in female patients, in subjects receiving bee venom vaccines, and in patients undergoing a rapid incremental phase, but not in patients with a history of a severe original SR (5). Similar findings for several of these parameters were reported in a previous, larger retrospective study that reported SRs were most likely to occur at venom doses between 1 and 50 µg and at maintenance dosage, and that severe anaphylactic reactions were more frequent in patients receiving beta-blockers (4). Beta-blockers do not appear to increase the overall risk of a SR during VIT, but in the case of a SR the event is likely to be more severe (1). In a recent review article that evaluated eight VIT studies in the general population, honeybee VIT was confirmed to be a risk factor for SR; side effects occurred in 26.6% of patients treated with honeybee venom, and in 11.2% of patients treated with yellow jacket venom (6). In contrast to the findings of the European multicenter study, several retrospective studies concluded that treatment with rush and ultrarush protocols are tolerated at least as well as or even better than treatment with slower protocols (7). Side effects during VIT are probably more frequent in mastocytosis patients, especially in those with a yellow jacket venom allergy (6). However, risk factors for adverse reactions in this patient population (e.g. type of mastocytosis, protocol) require further evaluation (10). Preliminary data from a multicenter European prospective study of 1,000 patients with venom allergy show that an elevated baseline mast cell tryptase concentration represents a risk factor for side effects during VIT. Unlike inhalant allergen immunotherapy, patients with venom allergy who show high sensitivity in skin tests or high titres in sIgE laboratory tests do non seem to be at higher risk of developing side effects during VIT (11).

Premedication

Pre-treatment with an H1 antihistamine has been demonstrated to reduce the number and severity of large local reactions and mild systemic reactions to VIT, such as urticaria and angioedema. Antihistamine pre-treatment should be started one or two days before VIT and continued until the maintenance dose has been well tolerated at least three times (1). In one study, antihistamine premedication with levocetirizine was reported to enhance the clinical efficacy of ultrarush honeybee VIT (12). However, this finding was not confirmed by another recent study, although levocetirizine was able to affect the expression of histamine receptors and cytokine production by allergen-specific T cells (13). Pre-treatment with a combination of H1 antihistamine and a corticosteroid have not been performed in honeybee and vespid VIT, except in patients with mast cell diseases (6). In a prospective, double-blind, randomised, placebo-controlled pilot study, the occurrence of local reactions after VIT was

significantly delayed by pre-treatment with the leukotriene antagonist montelukast (14). Pre-treatment with anti-IgE monoclonal antibodies may permit more rapid and higher doses of allergen immunotherapy while improving its safety. Moreover, this pre-treatment could play an important role in insect venom allergic patients who are intolerant to VIT. There are several case reports of bee venom allergic patients (15), including patients suffering from indolent systemic mastocytosis (16), who experienced SRs to VIT and who were able to tolerate VIT after pre-treatment with omalizumab. However, Soriano Gomis and colleagues reported a case of systemic allergic reaction with bee-venom immunotherapy in spite of pre-treatment with omalizumab and antihistamines (17). Further studies on larger patient populations are required to ascertain whether treatment with anti-IgE can enhanced the safety and perhaps efficacy of VIT.

Route of administration

Due to its good safety profile, sublingual immunotherapy (SLIT) is increasingly being used in European countries in patients with allergic asthma or rhinoconjunctivitis. In a placebo-controlled double-blind study on bee venom SLIT in patients with a history of LLRs, the diameter of LLR to a bee sting challenge was reduced by more than 50% in 57% of active treated patients (18). The authors concluded that more information on SRs is needed. However, on the basis of these findings alone (a partial or complete treatment failure in 43% of patients with LLRs) and without experimental data on the pharmacokinetics of venom SLIT, caution should be exercised when considering SLIT as a therapeutic option for patients with severe SRs (19). Intralymphatic administration of allergen has recently been investigated in specific immunotherapy for grass pollen allergic rhinitis (20). Compared with a 3-year course of conventional subcutaneous immunotherapy, intralymphatic administration enhanced the safety and efficacy of immunotherapy and reduced the treatment time from 3 years to 8 weeks (20). The therapeutic potential of bee venom intralymphatic immunisation has been investigated in sensitised mice using a model of anaphylaxis (21). Intralymphatic administration was associated with a much stronger increase in Th1-dependant protecting IgG2a antibodies, leading the authors to conclude that this approach could improve both the efficacy and safety of VIT (21). Current strategies to minimise the side effects associated with VIT are summarised in Table 2.

Table 2. Reducing side effects of VIT

Development of predictive tests
Defining the risk factors for SRs to VIT
Premedication
• Antihistamines
• Montelukast
• Omalizumab
Route of administration
• Sublingual
• Intralymphatic
Purified and depot extracts
Modified allergens

SR: systemic reaction
VIT: venom-specific immunotherapy

Purified and depot extracts

In Europe, purified or non-purified aqueous venom extracts are commercially available for ultrarush, rush, clustered and maintenance phases, with aluminium hydroxide adsorbed (so called depot) preparations used for the conventional up-dosing and maintenance phases. Many European specialists switch to depot preparations after up-dosing (1, 10). In general, the frequency of side effects during a conventional dose increase was lower in protocols that were carried out with depot extracts than in those that used aqueous extracts (7). Studies comparing purified aqueous extracts and depot preparations in VIT in honeybee and Vespid allergic patients, using different protocols, demonstrated that depot preparations were better tolerated than purified aqueous extracts and resulted in a lower frequency of LLRs and SRs (22-24). The therapeutic efficacy to in-field sting or sting challenge, however, was no different (22). A recent study compared the safety and tolerability of VIT with purified ex-

tracts and non-purified products in yellow jacket and honeybee allergic patients. The induction phase was carried out using a 2-7 day ultrarush protocol and results showed that VIT with purified extracts resulted in a significantly lower number of severe LLRs compared with VIT using non-purified preparations (25). At present, compared with therapy with an aqueous extract (both purified and non-purified), VIT with a depot extract is superior with respect to the frequency of occurrence of LLRs and SRs, and purified extracts appear to be safer than non-purified, especially with respect to the frequency of LLRs. Therefore, if protection by VIT is to be achieved rapidly, the use of purified preparations is preferred for rush treatment with the use of depot preparations for maintenance therapy.

Conclusion

VIT is highly effective but can be associated with side effects. However, only a few SRs induced by VIT are severe, and the large majority of patients tolerate treatment without any side effects. To limit the occurrence of SR to VIT, it is necessary to improve our knowledge of the risk factors. A number of promising new strategies that can improve efficacy and limit the side effects of VIT have recently been described and we eagerly await their application in clinical practice.

Disclosure

The authors state no conflicts of interest.

References

1. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;**60**:1459-1470.
2. Moffitt JE, Golden DB, Reisman RE, Lee R, Nicklas R, Freeman T et al. Stinging insect hypersensitivity: a practice parameter update. J Allergy Clin Immunol 2004;**114**:869-86.
3. Muller UR. Insect sting allergy. Clinical picture, diagnosis and treatment. Stuttgart-New York;Gustav Fischer Verlag 1990.
4. Lockey RF, Turkeltaub PC, Olive ES, Hubbard JM, Baird-Warren IA, Bukantz SC. The Hymenoptera venom study. III. Safety of venom immunotherapy. J Allergy Clin Immunol 1990;**86**:775-780
5. Mosbech H, Muller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. European Academy of Allergology and Clinical Immunology. Allergy 2000;**55**:1005-1010.
6. Niedoszytko M, de Monchy J, van Doormaal JJ, Jassem E, Oude Elberink JN. Mastocytosis and insect venom allergy: diagnosis, safety and efficacy of venom immunotherapy. Allergy 2009;**64**:1685.
7. Rueff F, Przybilla B: Venom immunotherapy: adverse reactions and treatment failure. Curr Opin Allergy Clin Immunol 2004;**4**:307-311.
8. Winther L, Arnved J, Malling HJ, Nolte H, Mosbech H. Side-effects of allergen-specific immunotherapy: a prospective multicentre study. Clin Exp Allergy 2006;**36**:254-260
9. Kosnik M, Silar M, Bajrovic N, Music E, Korosec P. High sensitivity of basophils predicts side-effects in venom immunotherapy. Allergy 2005;**60**:1401-1406.
10. Bilò MB, Bonifazi F. Advances in Hymenoptera venom immunotherapy. Curr Opin Allergy Clin Immunol 2007;**7**:567-573.
11. Birnbaum J, Ramadour M, Magnan A, Vervloet D. Hymenoptera ultra-rush venom immunotherapy (210 min): a safety study and risk factors. Clin Exp Allergy 2003;**33**:58-64.
12. Müller U, Hari Y, Berchtold E. Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. J Allergy Clin Immunol 2001;**107**:81-86.
13. Müller UR, Jutel M, Reimers A, Zumkehr J, Huber C, Kriegel C et al. Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy. J Allergy Clin Immunol 2008;**122**:1001-1007.
14. Wöhrl S, Gamper S, Hemmer W, Heinze G, Stingl G, Kinaciyan T. Premedication with montelukast reduces local reactions of allergen immunotherapy. Int Arch Allergy Immunol 2007;**144**:137-142.

15. Galera C, Soohun N, Zankar N, Caimmi S, Gallen C, Demoly P. Severe anaphylaxis to bee venom immunotherapy: efficacy of pretreatment and concurrent treatment with omalizumab. J Investig Allergol Clin Immunol 2009;**19**:225-229.
16. Kontou-Fili K. High omalizumab dose controls recurrent reactions to venom immunotherapy in indolent systemic mastocytosis. Allergy 2008;**63**:376-378.
17. Soriano Gomis V, Gonzalez Delgado P, Niveiro Hernandez E. Failure of omalizumab treatment after recurrent systemic reactions to bee-venom immunotherapy. J Investig Allergol Clin Immunol 2008;**18**:225-256.
18. Severino MG, Cortellini G, Bonadonna P, Francescato E, Panzini I, Macchia D et al. Sublingual immunotherapy for large local reactions caused by honey bee stings. J Allergy Clin Immunol 2008;**122**:44-88.
19. Ruëff F, Bilò MB, Jutel M, Mosbech H, Müller U, Przybilla B; Interest group on Hymenoptera venom allergy of the European Academy of Allergology and Clinical Immunology. Sublingual immunotherapy with venom is not recommended for patients with Hymenoptera venom allergy. J Allergy Clin Immunol 2009;**123**:272-273.
20. Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. Proc Natl Acad Sci U S A 2008;**105**:17908-17912.
21. Martínez-Gómez JM, Johansen P, Erdmann I, Senti G, Crameri R, Kündig TM. Intralymphatic Injections as a New Administration Route for Allergen-Specific Immunotherapy. Int Arch Allergy Immunol 2009;**150**:59-65.
22. Ruëff F, Wolf H, Schnitker J, Ring J, Przybilla B. Specific immunotherapy inoneybee venom allergy: A comparative study using aqueous and aluminium hydroxide adsorbed preparations. Allergy 2004;**59**:589-595.
23. Quercia O, Emiliani F, Pecora S, Burastero SE, Stefanini GF. Efficacy, safety, and modulation of immunologic markers by immunotherapy with honeybee venom: Comparison of standardized quality depot versus aqueous extract. Allergy Asthma Proc 2006;**27**:151-158.
24. Alessandrini AE, Berra D, Rizzini FL, Mauro M, Melchiorre A, Rossi F et al. Flexible approaches in the design of subcutaneous immunotherapy protocols for hymenoptera venom allergy. Ann Allergy Asthma Immunol 2006;**97**:92-97.
25. Bilò MB, Severino M, Cilia M, Pio A, Casino G, Ferrarini E et al. The VISYT trial: Venom Immunotherapy Safety and Tolerability with purified vs nonpurified extracts. Ann Allergy Asthma Immunol 2009;**103**:57-61.

MONITORING VENOM IMMUNOTHERAPY

Franziska Ruëff

Klinik und Poliklinik für Dermatologie und Allergologie, Ludwig-Maximilians-Universität, Munich, Germany

Venom immunotherapy (VIT) is a highly effective treatment for Hymenoptera venom allergy that offers the majority of patients protection against systemic reactions if restung by the culprit insect (Table 1). A few studies have reported that up to 30% of the patients on VIT are not protected. However, by increasing maintenance dose of venom to 200 µg or more, nearly all patients can be adequately protected against a subsequent reaction (7). In the case of treatment failure, there is a clear therapeutic consequence, therefore the identification of unprotected patients is important in order to optimise the results of VIT. A further reason to assure the efficacy of VIT is to provide the patient with reassurance that they will be protected against a subsequent severe reaction to a sting and will feel more at ease while outdoors.

It is desirable to have a test for the efficacy of VIT that does not harm the patient. Currently, the options for monitoring VIT include: skin tests, *in vitro* tests, and sting-challenge tests. Many *in vitro* parameters as well as skin test reactivity change during the course of VIT; however, similar to the pre-treatment situation, there is no *in vitro* parameter, which reliably indicates the clinical reactivity of a sensitised patient. The relative advantages and disadvantages of the different tests are discussed below.

Table 1. Sting challenge in patients on immunotherapy with 100 µg of the respective venom

Reference	n	BV / VV	Systemic reaction (%)
Golden, 1981 (1)	147	+ / +	2.7
Urbanek, 1985 (2)	66	66 / -	6.1 / -
Przybilla, 1987 (3)	157	+ / +	20.4
Müller, 1992 (4)	205	148 / 57	23.0 / 8.8
Haeberli, 2003 (5)	161	104 / 57	32.7 / 12.3
Ruëff, 2004 (6)	49	49 / -	16.3 / -

BV, treatment with honeybee; VV, treatment with vespid venom

Skin tests
A patient's reactivity to a skin test declines during VIT (4, 8-10). This effect may be attributed to a factor present in the patient's serum; serum of patients on VIT is able to neutralise skin reactions to wasp venom extracts *in vivo* (11). This ability to neutralise skin test reactivity is correlated with the duration of VIT as well as with the concentration of venom-specific IgG and IgG4 (11). However, a lower skin test reactivity compared with pre-treatment reactivity in patients on VIT does not reliably indicate protection at a re-sting.

***In vitro* tests**
Venom-specific IgE increases at the start of VIT and declines with continued treatment (6, 11, 12), whereas venom-specific IgG increases after several months to years and remains high at least as long VIT is continued (6, 11, 12). The ratio of venom-specific IgE/IgG4 first increases then later decreases during VIT (11). Not only the serum concentration but also the pattern of specific IgE and IgG4 changes: on immunoblots, some bands for single venom allergens may disappear or decrease, and new IgG4 antibodies can appear (13).

Cellular tests measure basophil activation upon stimulation with venom. Reduced upregulation of the activation marker CD63 was apparent during VIT, and in 60% of patients who received VIT with vespid venom the basophil activation test was negative after three years (14). After rush VIT, venom induced histamine and sulfidoleukotriene release are immediately reduced (15). These effects were shown to be in part mediated by IFN-gamma and IL-10 production (15). VIT changes allergen-specific T-cell reactivity; shortly after the start of VIT, IL-4 and IL-5 were decreased and secretion of IFN-gamma from venom-stimulated T-cell cultures was increased (12, 16). Recently, a small, but continuous decrease in baseline tryptase concentration over time was reported, which correlated with the duration of VIT (17). This decline may indicate a dampened mast cell function or decline in mast cell burden.

Several of these effects are interesting with respect to immunologic events that occur during VIT. Previously, some of these parameters (e.g. venom-specific IgE) were also recommended as indicators for deciding when to stop VIT (18). However, in a more recent EAACI position paper, the results of skin and in vitro tests for the prognosis of patients on or after VIT played a more minor role (19). This change in the interpretation of laboratory findings was a result of various studies that examined the diagnostic value of laboratory parameters in patients on VIT who underwent diagnostic sting challenge or were accidentally stung. The findings indicated that all laboratory parameters were unsuccessful in assessing the efficacy of VIT:

- *In vitro* findings from 15 patients with and 52 patients without systemic adverse reaction (SAR) at sting challenge were compared (8). There was no difference between both groups with respect to venom-specific IgE. Moreover, the IgG/IgE ratio was higher in patients with SAR at sting challenge.
- 17 subjects who tolerated a field sting showed no difference in the pattern of venom-specific IgE and IgG4 compared with 14 patients who continued to experience systemic reactions to stings despite VIT (20).
- Venom-induced leukotriene release from peripheral blood leukocytes was investigated before VIT and after a tolerated field sting; no reduction of leukotriene release compared with pre-treatment values was observed in 14 subjects (21).
- No change in basophil CD63-expression compared with pre-treatment values was observed in 20 vespid venom-allergic patients who received VIT and who already tolerated a sting challenge (22). However, a basophil activation test showed a higher reactivity in 14 patients who still reacted to bee or wasp sting compared with 17 patients who tolerated field stings (20).
- In 362 patients, there was no correlation between systemic reaction at sting challenge and a lack of VIT-induced venom-specific IgG4 to major Hymenoptera allergens (23).

Despite promising results, which suggest that *in vitro* tests might have a role for mon-

itoring efficacy of VIT, studies including larger numbers of patients in whom a sting challenge test was performed are required to confirm these findings. Thus, despite the availability of new laboratory techniques, these still do not allow to predict the clinical reactivity of patients on VIT.

Sting challenge test

Challenge tests aim to reveal the clinical reactivity of an individual to defined allergen exposure. Some aspects of the sting challenge test with insect venom differ from challenge tests with other allergens: (i) available standardised venom extracts are not suitable for sting challenge tests (24); (ii) incremental doses of venom cannot be applied; and (iii) the exact dose injected by an insect sting is not clear. There are also additional problems: during the warm season, it is not a problem to obtain living honeybees for sting challenge tests, however, this can be more problematic for vespids. Correct identification of the insects is also important, as the species of vespid will influence the outcome of the sting challenge test (25).

Before performing a sting challenge, a number of technical aspects and precautionary measures must be considered. These have been described elsewhere in detail (26), however, the most important measures are listed in Table 2. Sting challenge tests can only be carried out in an environment where the necessary trained personnel and equipment for the treatment of anaphylactic reactions are available (27). Nevertheless, it should be borne in mind that over 50% of accidentally occurring fatal drug reactions take place in operating theatres (28). Thus, despite optimal conditions for patient management, patients may die as a result of an anaphylactic reaction. Patient selection is therefore clearly important, and if the risk of a severe reaction to a sting challenge is high, the patient should first be treated with an increased maintenance dose of venom before a sting challenge. If this procedure is followed, severe reactions are very rare. Amongst the author`s own patients (n=1071) 1204 sting challenge tests were evaluated between 1999 and 2007. Subjective- and objective-systemic reactions occurred in 0.8% and 7.7% of the tests respectively. These were severe (anaphylactic shock) in five patients, and adrenaline treatment was necessary in four of these cases, all patients recovered without sequelae.

The prognostic reliability of a tolerated sting challenge was close to 100% in a limited number of repeatedly stung patients (2). However, some patients who tolerate a sting challenge may still experience a reaction to a subsequent sting. Repeated sting challenges performed one year after a first tolerated sting indicated that 21% of patients had a systemic reaction to the second sting ex-

Table 2. Practical performance of the sting challenge test (26)

- VIT tolerated
- If patient experienced a systemic reaction to a field sting - dose increase
- Patient disposed to intubation (fasting)
- No relevant current diseases
- No ACE inhibitors or beta-blockers*
- Written informed consent
- Intravenous line
- Monitoring time >2 hours or longer, depending on the patient's history (preferably overnight)
- Emergency standby (personnel, equipment)
- Monitoring of all subjective or objective signs
- In patients who experience systemic reactions, immediate treatment of symptoms according to the recommendations of the guidelines. Overnight surveillance in hospital after recovery

* There are no data supporting the hypotheses that beta-blockers can enhance the risk for sting reactions in patients on VIT (30). However, for security reasons the withdrawal of beta-blockers (including eye drops) before challenge tests is recommended.

posure (29). Sting challenge tests are not recommended for individuals who are not being treated with VIT (26), and are also not recommended as a routine diagnostic method for patients who have stopped VIT, as these stings might boost already decreased sensitisation or even re-sensitise the patient. In conclusion, sting challenges in patients on maintenance VIT are recommended to identify those who are not protected. A sting challenge test is particularly important in those patients who are at increased risk due to intense exposure or due to their susceptibility to severe anaphylactic reactions. Full protection against a severe reaction can nearly always be achieved by increasing the venom maintenance dose (7).

Disclosure

The author states no conflicts of interest.

References

1. Golden DB, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Dose dependence of Hymenoptera venom immunotherapy. J Allergy Clin Immunol 1981;**67**:370-374.
2. Urbanek R, Forster J, Kuhn W, Ziupa J. Discontinuation of bee venom immunotherapy in children and adolescents. J Pediatr 1985;**107**:367-371.
3. Przybilla B, Ring J, Grießhammer B, Braun-Falco O. Schnellhyposensibilisierung mit Hymenopterengiften. Verträglichkeit und Therapieerfolg. Dtsch Med Wochenschr 1987;**112**:416-424.
4. Müller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. J Allergy Clin Immunol 1992;**89**:529-535.
5. Haeberli G, Brönnimann M, Hunziker T, Müller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003;**33**:1216-1220.
6. Ruëff F, Wolf H, Schnitker J, Ring J, Przybilla B. Specific immunotherapy in honeybee venom allergy: a comparative study using aqueous and aluminium hydroxide adsorbed preparations. Allergy 2004;**59**:589-595.
7. Ruëff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving Hymenoptera venom immunotherapy are protected by increased venom doses. J Allergy and Clin Immunol 2001;**108**:1027-1032.
8. Müller U, Helbling A, Bischof M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. Allergy 1989;**44**:412-418.
9. de Jong NW, Vermeulen AM, de Groot H. Allergy to bumblebee venom. III. Immunotherapy follow-up study (safety and efficacy) in patients with occupational bumblebee-venom anaphylaxis. Allergy 1999;**54**:980-984.
10. Quercia O, Emiliani F, Pecora S, Burastero SE, Stefanini GF. Efficacy, safety, and modulation of immunologic markers by immunotherapy with honeybee venom: comparison of standardized quality depot versus aqueous extract. Allergy Asthma Proc 2006;**27**:151-158.
11. Senti G, Johansen P, Oliver R, Prinz Vavricka BM, Graf N, Wuthrich B et al. A cutaneous allergen neutralisation test that correlates with the duration of venom immunotherapy. Int Arch Allergy Immunol 2006;**141**:377-383.
12. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Müller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. J Immunol 1995;**154**:4187-4194.
13. Pereira Santos MC, Pedro E, Spínola Santos A, Branco Ferreira M, Palma Carlos ML, Palma Carlos AG. Immunoblot studies in allergic patients to hymenoptera venom before and during immunotherapy. Eur Ann Allergy Clin Immunol 2005;**37**:273-278.
14. Ebo DG, Hagendorens MM, Schuerwegh AJ, Beirens LM, Bridts CH, De Clerck LS et al. Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. Cytometry B Clin Cytom 2007;**72**:196-203.

15. Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-gamma production of T cells. J Allergy Clin Immunol 1999;**103**:326-332.
16. McHugh SM, Deighton J, Stewart AG, Lachmann PJ, Ewan PW. Bee venom immunotherapy induces a shift in cytokine responses from a TH-2 to a TH-1 dominant pattern: comparison of rush and conventional immunotherapy. Clin Exp Allergy 1995;**25**:828-838.
17. Dugas-Breit S, Przybilla B, Dugas M, Arnold A, Pfundstein G, Küchenhoff H et al. Serum concentration of baseline mast cell tryptase: evidence for a decline during long-term immunotherapy for Hymenoptera venom allergy Clin Exp Allergy. In press.
18. Müller U, Mosbech H. Position paper: Immunotherapy with Hymenoptera venoms. Allergy 1993;**48**:36-46.
19. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;**60**:1459-1457.
20. Peternelj A, Silar M, Erzen R, Kosnik M, Korosec P. Basophil sensitivity in patients not responding to venom immunotherapy. Int Arch Allergy Immunol 2008;**146**:248-254.
21. Borelli S, Anliker M, Bucher C, Wüthrich B. CAST-ELISA for monitoring specific venom immunotherapy: final results of a three year prospective study. Allergo J 2003;**12**:S69-70.
22. Erdmann S, Sachs B, Moll-Slodowy, Merk H. CD63 expression on basophils in the diagnosis of wasp venom allergy and for monitoring specific immunotherapy. Allergo J 2003;**12**:S55-57.
23. Ollert M, Ruëff F, Przybilla B, Müller U, Ring J. Results of a multicenter study on the prognostic value of immunoblot tests in patients on Hymenoptera venom immunotherapy. Allergo J 2003;**12**:S71-72.
24. Schultze-Werninghaus C, Wahn U, Niggemann B. Evaluation of the risk of anaphylactic reactions by wasp venom-extract challenges in children. Pediatr Allergy Immunol 1999;**10**:133-137.
25. Golden DB, Breisch NL, Hamilton RG, Guralnick MW, Greene A, Craig TJ et al. Clinical and entomological factors influence the outcome of sting challenge studies. J Allergy Clin Immunol 2006;**117**:670-675.
26. Ruëff F, Przybilla B, Müller U, Mosbech H. The sting challenge test in Hymenoptera venom allergy. Allergy 1996;**51**:216-225.
27. Alrasbi M, Sheikh A. Comparison of International guidelines for the emergency medical management of anaphylaxis. Allergy 2007;**62**:838-841.
28. Pumphrey RS. Lessons for management of anaphylaxis from a study of fatal reactions. Clin Exp Allergy 2000;**30**:1144-1150.
29. Franken HH, Dubois AEJ, Minkema HJ, van der Heide S, de Monchy JGR. Lack of reproducibility of a single negative sting challenge response in the assessment of anaphylactic risk in patients with suspected yellow jacket hypersensitivity. J Allergy Clin Immunol 1994;**93**:431-436.
30. Müller U, Haeberli G. Use of beta-blockers during immunotherapy for Hymenoptera venom allergy. J Allergy Clin Immunol 2005;**115**:606-610.

VENOM IMMUNOTHERAPY IN DIFFICULT-TO-TREAT PATIENTS

Patrizia Bonadonna

Allergy Unit Verona General Hospital, Verona, Italy

In clinical practice the optimal conditions for treating a patient with Hymenoptera venom allergy rarely arise. In reality, many patients are being treated with concomitant medications that can increase the risk of side effects during venom immunotherapy (VIT) or may suffer from a disease that contraindicates VIT. In addition, risk factors, such as the age of the patient can also play a role in the decision to treat a patient with VIT, and in the success of the treatment. Here we discuss some factors that can affect the outcome of VIT.

Mastocytosis

Mastocytosis is characterised by abnormal proliferation and maturation of mast cells in the skin, or in bone marrow, liver, spleen, lymph nodes, or other tissues. Clinically this can present as a wide variety of conditions, ranging from asymptomatic indolent systemic mastocytosis to the aggressive systemic form of the disease. Accumulation of mast cells is caused by mutations in the c-kit gene, which codes for the stem cell factor receptor on the surface of mast cells. This mutation causes the receptor activate in the absence of ligand and leads to mast cell proliferation (1). In adults with systemic mastocytosis, the predominant mutation is the substitution of an asparagine for a valine residue at position 816 (D816V) (1). According to the WHO classification, mastocytosis can be sub-classified as: cutaneous mastocytosis, when the disease is limited to the skin; and systemic mastocytosis, when mast cells infiltrate one or more extracutaneous organs. The most frequent type of cutaneous mastocytosis is urticaria pigmentosa (2). Urticaria pigmentosa consists of multiple pigmented lesions, typically less than 5 mm in diameter, however individual lesions may be larger in children.

The diagnosis of systemic mastocytosis requires satisfaction of both the major criteria plus one minor criterion, or alternatively three minor criteria. Major criteria are: multifocal dense infiltrates of mast cells in bone marrow, or in other extra-cutaneous organs. Minor criteria include: abnormal morphology of extra-cutaneous mast cells; increased serum tryptase level (>20 ng/mL); mutation in the kit proto-oncogene at codon 816 in an extra-cutaneous organ; and expression of CD2 and/or CD25 on bone marrow mast cells (2). The term monoclonal mast cell activation syndrome, has been proposed for subjects with unexplained recurrent anaphylaxis without skin lesions who only meet one (excluding raised tryptase level) or two of the minor criteria for systemic mastocytosis. Although aggressive and malignant forms of the disease occur, the most common form associated with Hymenoptera allergy is indolent systemic mastocytosis (3). This form can be differentiated from severe forms of systemic mastocytosis by a lack of evidence of

an associated clonal haematological non-mast cell disorder and lack of one organ dysfunction or lack of evidence of mast-cell leukemia. In systemic mastocytosis most of the clinical symptoms are due to release of mast-cell mediators such as histamine and tryptase. The mediator release in mastocytosis can occur through a non-IgE mediated mechanism and be triggered by: physical stimuli, such as temperature change, pressure or exercise, and selected medications, including opioids, anesthetic agents, aspirin and radio contrast media (4). These mediators affect the skin, causing pruritus and flushing; the gastrointestinal tract, causing nausea, abdominal pain/cramps, diarrhoea and gastrointestinal ulcers; and the cardiovascular system, where they cause fatigue, dizziness, palpitation, hypotension and in some cases, anaphylactic shock. In addition the release of mediators from mast cells can lead to bone and soft tissue pain, and neuropsychiatric symptoms, including headaches, cognitive problems, and paraesthesia. Besides these mediator-induced symptoms, certain clinical manifestations can also be due to mast cell infiltration such as: osteoporosis with pathological fractures, cytopenia, hepatospenomegaly, malabsorption, limphadenopathy and other organ dysfunction. Elevated total serum tryptase level is therefore highly indicative of mast cell degranulation as seen after systemic anaphylaxis. Baseline serum tryptase level correlates with the total number mast cells and is therefore increased in patients with mastocytosis.

Mast cells can be also be activated by an IgE-mediated reaction, such as occurs in hymenoptera venom allergy. Hymenoptera venom allergy is the most common cause of anaphylaxis in subjects with mastocytosis (5). Subjects with systemic mastocytosis and Hymenoptera venom allergy have an increased risk of severe systemic reactions, compared to subjects without systemic mastocytosis. This has been described in several case reports and small series studies of subjects with Hymenoptera venom allergy (6-8). The incidence of mastocytosis in the general population is less than 1/100,000, but is significantly higher in patients with Hymenoptera venom allergy. Different studies, using different diagnostic protocols have estimated this at between 1% and 5.8% (9-11). The higher incidence of 5.8% observed in the author's own study could be explained by the diagnostic protocol which involved bone marrow biopsy and aspiration, flow cytometric analysis, c-kit mutation, and a cutoff value for serum baseline total tryptase level of 11.4 ng/mL instead of the 20 ng/mL recommended in the WHO guidelines. The diagnostic work-up for systemic mastocytosis is usually limited to patients with typical skin lesions, because cutaneous mastocytosis or systemic mastocytosis with skin involvement is believed to represent the majority of the patients with Hymenoptera venom allergy. Instead, González de Olano and collegues reported that almost 20% of subjects with Hymenoptera venom allergy and systemic mastocytosis do not have skin involvement (12).

The assessment of baseline serum tryptase levels is an inexpensive, reliable and simple screening test for mastocytosis in subjects with a positive history of systemic reaction to Hymenoptera stings. Moreover, it may be useful to routinely measure serum tryptase level in patients with Hymenoptera venom allergy, especially in those without skin involvement. A cut-off value of 11.4 ng/mL for baseline serum tryptase concentration increases the sensitivity for the diagnosis of clonal mast cell disorders. It should also be borne in mind that subjects who test negative for Hymenoptera venom allergy and experience anaphylaxis after wasp or bee stings may have undiagnosed mastocytosis.

Immunotherapy

The treatment of choice in patients with Hymenoptera venom allergy is VIT, however, its use in patients with systemic mastocytosis remains controversial. Three recent studies showed that VIT was not only well tolerated by most patients with mastocytosis but also protects most from further anaphylactic sting reactions (8, 11, 12). Although most patients with mastocytosis are protected by VIT, a minority may develop systemic allergic symp-

toms after a re-sting. In this case it is recommended to increase the maintenance dose to 200 µg and to continue VIT for life.
Although most patients with systemic mastocytosis tolerate VIT without problems, some may develop severe and recurrent side effects from VIT that make it difficult to reach the recommended maintenance dose of 100 µg. It is therefore recommended that premedication with an antihistamine be given during the dose-increase phase. A recent report described successful prophylactic treatment with omalizumab that enabled the patient to reach the maintenance dose without experiencing further side effects (13)

Other risk factors

Other risk factors also play a role in the outcome of VIT. Fatality caused by anaphylactic shock from Hymenoptera allergy is higher in the elderly. This is in contrast to death due to anaphylactic shock from food allergy, which occurs mainly in younger atopic individuals with bronchial asthma. This is likely due to the fact that older people often have a pre-existing cardiovascular disease that constitutes a risk factor for fatal anaphylactic reactions. During the anaphylactic reaction, symptoms like arterial hypotension, cardiovascular collapse, arrhythmia or chest pain may cause worsening of a pre-existing cardiovascular disease. Elderly people are also more likely to be on treatment with beta-blockers. It is well known that immunotherapy may cause anaphylactic side effects and there have been reports of concomitant beta-blockers aggravating an anaphylactic reaction and complicating treatment. For this reason beta-blockers are generally not recommended in patients with asthma and those on allergen immunotherapy. However beta-blockers are widely used in treatment of cardiovascular diseases, such as coronary heart disease, chronic heart failure and ventricular arrhythmia and are extremely valuable and difficult to replace. This raises the question of what to do in these cases? A retrospective analysis by Müller and coworkers on patients with cardiovascular diseases, with and without beta-blockers, did not find a higher incidence of systemic allergic reactions following injections during VIT (14). In addition, the reactions that did occur were not more severe than in the control group, and patients receiving beta-blockers were able to continue VIT. Systemic reactions after re-exposure during VIT were neither more frequent nor more severe in patients treated with beta-blockers. As a result, a position paper of hymenoptera venom allergy from the European Academy of Allergy and Clinical Immunology proposed that the contraindication for the use of beta-blockers during VIT should not be considered absolute (15).
Replacement of beta-blockers must be evaluated carefully in patients with Hymenoptera venom allergy. For patients with a high degree of sting exposure such as beekeepers, farmers or gardeners and who have a cardiovascular disease, VIT could be started under beta-blockers. Patients who take beta-blockers for hypertension without other cardiac diseases, may have beta-blockers replaced by other anti-hypertensives, such as calcium antagonists, and/or diuretics. Finally, if a patient has only a marginal risk of a re-sting and has a cardiovascular disease that requires use of beta-blockers, the option to continue treatment with beta-blockers, and not to perform VIT and supply emergency medications, should be considered.
Angiotensin Converting Enzyme (ACE) inhibitors have been associated with severe allergic reactions in individuals who were stung or received VIT (16, 17). This it thought to result from a decreased concentration of angiotensin II further reducing the ability to counteract vasodilation. ACE inhibitors also interfere with the kallikrein-kinin system and could result in an accumulation of bradikinin, that promotes vasodilatation and increase vascular permeability. Therefore, ACE inhibitor therapy should be avoided where possible, or temporarily discontinued prior to each venom injection. However, in light of the beneficial effects on mortality and morbidity in diseases such as congestive heart failure and renal disease, ACE inhibitor therapy may be deemed necessary in a given patient. In patients where ACE inhibitor therapy is necessary and where VIT is indicated, temporary discontinuation of the ACE inhibitor 24 hours

before to venom injections has been suggested. ACE inhibitors with active metabolites that have prolonged terminal half-lives (*e.g. benazepril, quinapril, ramipril*) may require discontinuation for longer time periods prior to VIT injection. A recent review of a population that included patients receiving both VIT and ACE inhibitors did not find an increased risk in these patients. Thirteen of 62 experienced a systemic reaction (SR) during VIT, compared with thirteen of patients not taking an ACE inhibitor (18). The authors concluded that there was no association between the use of ACE inhibitors and increased frequency of SRs to VIT. Nevertheless the safety of ACE inhibitors use in patients receiving VIT requires further study.

In conclusion, several concomitant factors can affect the tolerability and outcome of VIT. These should be taken into account by the physician and the use of prophylactic medication, suspension of patients medication, and modification of the VIT protocol should be based on an individual benefit-to-risk assessment for the patient.

Disclosure

The author states no conflicts of interest.

References

1. Bunimovich O, Grassi M, Baer MR. Systemic mastocytosis: classification, pathogenesis, diagnosis, and treatment. Cutis 2009;**83**:29-36.
2. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organisation criteria and point-of-care diagnostic algorithms. Leukemia 2008;**22**:14-22.
3. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal Fior D et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol 2009;**123**:680-6.
4. Brockow K, Romano A, Aberer W, Bircher AJ, Barbaud A, Bonadonna P et al.; European Network of Drug Allergy and the EAACI interest group on drug hypersensitivity. Skin testing in patients with hypersensitivity reactions to iodinated contrast media - a European multicenter study. Allergy 2009;**64**:234-241.
5. Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. Allergy 2008;**63**:226-232.
6. Müller UR, Horat W, Wüthrich B, Conroy M, Reisman RE. Anaphylaxis after Hymenoptera stings in three patients with urticaria pigmentosa. J Allergy Clin Immunol 1983;**72**:685-689.
7. Oude Elberink JN, de Monchy JG, Kors JW, van Doormaal JJ, Dubois AE. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two patients with mastocytosis. J Allergy Clin Immunol 1997;**99**:153-154.
8. Fricker M, Helbling A, Schwartz L, Müller U. Hymenoptera sting anaphylaxis and urticaria pigmentosa: clinical findings and results of venom immunotherapy in ten patients. J Allergy Clin Immunol 1997;**100**:11-15.
9. Dubois AE. Mastocytosis and Hymenoptera allergy. Curr Opin Allergy Clin Immunol 2004;**4**:291-295.
10. Ruëff F, Placzek M, Przybilla B. Mastocytosis and Hymenoptera venom allergy. Curr Opin Allergy Clin Immunol 2006;**6**:284-288.
11. Bonadonna P, Zanotti R, Pagani M, Caruso B, Perbellini O, Colarossi S et al. How much specific is the association between hymenoptera venom allergy and mastocytosis? Allergy 2009;**64**:1379-1382.
12. González de Olano D, Alvarez-Twose I, Esteban-López MI, Sánchez-Muñoz L, de Durana MD, Vega A et al. Safety and effectiveness of immunotherapy in patients with indolent systemic mastocytosis presenting with Hymenoptera venom anaphylaxis. J Allergy Clin Immunol 2008;**121**:519-526.
13. Kontou-Fili K, Filis CI. Prolonged high-dose omalizumab is required to control reactions to venom immunotherapy in mastocytosis. Allergy 2009;**64**:1384-1385.
14. Müller UR, Haeberli G. Use of beta-block-

ers during immunotherapy for Hymenoptera venom allergy. J Allergy Clin Immunol 2005;**115**:606-610.
15. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy. 2005;**60**:1459-1470
16. Ober AI, MacLean JA, Hannaway PJ. Life-threatening anaphylaxis to venom immunotherapy in a patient taking an angiotensin-converting enzyme inhibitor. J Allergy Clin Immunol 2003;**112**:1008-1009.
17. Stumpf JL, Shehab N, Patel AC. Safety of Angiotensin-converting enzyme inhibitors in patients with insect venom allergies. Ann Pharmacother 2006;**40**:699-703.
18. White KM, England RW. Safety of angiotensin-converting enzyme inhibitors while receiving venom immunotherapy. Ann Allergy Asthma Immunol 2008;**101**:426-430.

FUTURE STRATEGIES IN TREATMENT OF INSECT VENOM ALLERGY

Ulrich R. Müller

Allergiestation Medizinische, Klinik Ziegler Spital, Bern, Switzerland

Venom immunotherapy (VIT) is often cited as the most efficient form of specific allergen immunotherapy (1). Treatment is safe and protects most patients with venom allergy from further systemic allergic sting reactions. Why then should we look for future strategies? Here we present several aspects of VIT where significant improvements in treatment are possible and could be valuable.

Better selection of venoms for VIT

Specific IgE (sIgE) to both honeybee (BV) and *Vespula* venom (VV) is frequently observed in allergic subjects, despite limited cross-reactivity between the major protein allergens of the two venoms (50% sequence identity of the hyaluronidase) (2). This is especially true in countries with more BV than VV allergic patients, where more than 50% of patients with a history of systemic sting reactions have positive serum sIgE antibodies to both venoms. Most of the cross-reactivity is due to crossreacting carbohydrate determinants (CCDs) and is largely clinically insignificant (3). In a recent study that included 200 patients with a history of either honeybee or *Vespula* sting reactions, double positivity of sIgE was observed in 59% of patients (4). Immunotherapy using recombinant non-glycosylated major allergens, phospholipase A2 for BV (sIgE present in 99% of whole BV positive BV allergic pts) and antigen 5 for VV (sIgE present in 96% of whole VV positive VV allergic pts), reduced the frequency of double positivity to 17%. This approach will reduce the number of patients who need to undergo VIT with both venoms and thus significantly lower the cost of treatment.

Fewer injections during the maintenance phase

After reaching a maintenance dose of 100 µg, most patients are protected from a systemic reaction to a subsequent sting. According to European clinical guidelines further injections of the maintenance dose are recommended every four weeks during the first year of treatment, and every six weeks in subsequent years (5). Studies were carried out to investigate the effects of increasing the maintenance dose interval from the second year onward, these studies reported no difference with an interval of three months and reduced efficacy only when the interval was increased to six months (6, 7). It is possible, however, that the number of patients studied may have been too small to show up minor differences between the treatment schedules. It should be borne in mind that among beekeepers, those with less than ten stings a year are those who most often develop systemic allergic reactions (8).

Modification of allergens to increase efficacy and safety of VIT

Both the efficacy and safety of VIT are not as good as is often claimed, especially with bee venom, where 10 to 20% of treated patients develop some, mostly mild, systemic allergic symptoms when re-stung during immunotherapy, and 20 to 30% develop systemic allergic side effects, especially during rush and ultrarush protocols for dose increase (5). For this reason, various modified BV allergens, which showed diminished IgE binding but retained T-cell stimulating capacities were studied. Both mPEG (monomethoxypolyethylenglycol) - coupled BV and T-cell epitope peptides of the major BV allergen phospholipase A2 (PLA/Api m 1) showed strongly reduced IgE binding and were well tolerated during up-dosing. However, clinical efficacy, defined by the reaction to a sting challenge, was reduced (9,10). A chimeric protein containing six pieces of the three BV allergens PLA (Api m 1), hyaluronidase (Api m 2) and melittin (Api m 4), covering the whole peptide sequence of the three allergens, has been characterised *in vitro* (Fig. 1). The protein showed strongly reduced binding to IgE but maintained T-cell stimulating activity (11). However, the protein has not yet been studied clinically.

Modification of application route for VIT

Recently, sublingual immunotherapy (SLIT) has also been proposed for the treatment of venom allergy. In a placebo-controlled double-blind study on SLIT with BV, 26 patients with a history of large local reactions to honeybee stings and positive diagnostic tests for BV IgE were treated with SLIT using either BV or placebo (12). After six months, a significant reduction in the mean diameter of the local reaction to a bee sting challenge was observed in the venom, but not the placebo group. However, data on the preparation of the BV sac extract and on any side effects of the treatment were not given. From a theoretical basis SLIT for venom allergy does not appear to be a practical approach for two reasons: First, besides protein allergens, Hymenoptera venom contains a number of strongly cytotoxic peptides, some of them, like melittin, also have the capacity to bind IgE and cause heavy irritation of mucosal membranes. In order to use these venoms for SLIT it would first be necessary to remove these lower molecular weight peptides. This could reduce the effectiveness of the therapy as these peptides are also allergens. Second, venom allergy, in contrast to hay fever and asthma, is mediated via the skin and not mucosal membranes.

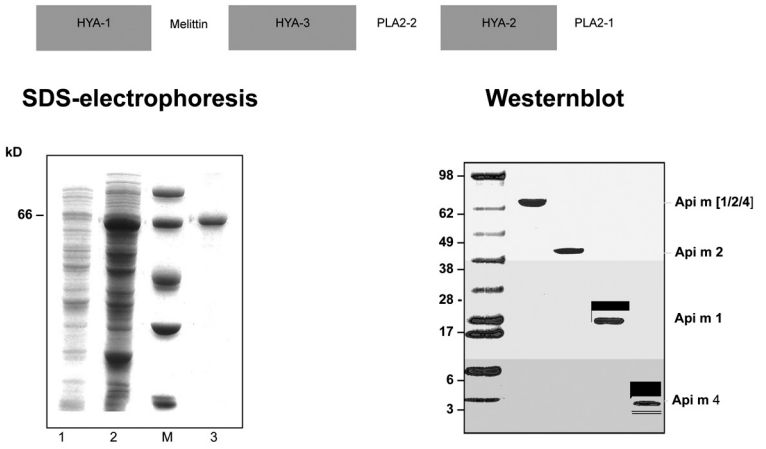


Fig. 1. Gene recombination of major allergens as a novel vaccine for allergen specific immunotherapy

Another group of researchers has proposed intralymphatic allergen administration for specific immunotherapy (13). In a controlled open label study 76 patients with seasonal allergic rhinitis to grass pollen were either treated by conventional subcutaneous immunotherapy (37 patients) over three years or by three injections in inguinal lymphnodes over two months (39 patients). The groups were compared repeatedly during the three-year study period using nasal provocation tests, symptom scores, skin tests and measures of specific IgE. While the long-term results were comparable between the groups, a decrease in the symptom score and nasal sensitivity occurred more rapidly in the group with intralymphatic administration. The intralymphatic administration group also experienced fewer side effects (Fig. 2).

The same researchers compared intralymphatic and subcutaneous application of BV-PLA for treatment of PLA-induced anaphylaxis in a mouse model (14). Intralymphatic administration was associated with a much stronger increase in Th1-dependant protecting IgG2a antibodies. The authors concluded that intralymphatic application could be safer and more efficient than conventional VIT and would enable a significant reduction in the number of injections. Similar studies in humans, including clinical parameters and sting challenges, are needed to assess the effectiveness of this route of allergen administration.

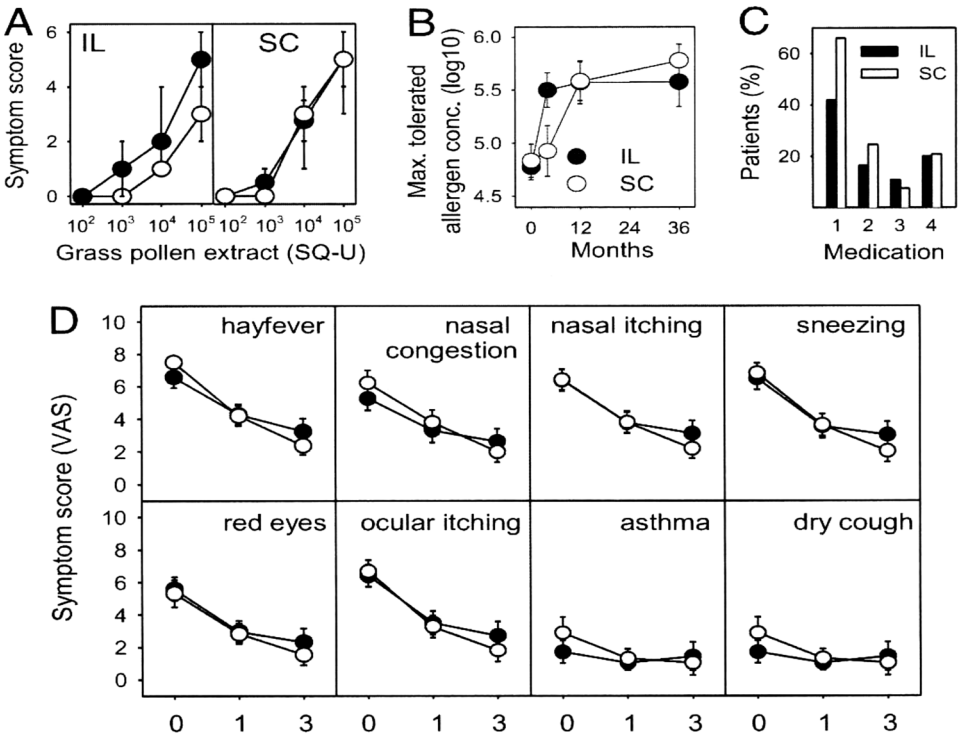


Fig. 2. Results from a randomised controlled trial of intralymphatic allergen administration. Reproduced from Senti G, Prinz Vavricka BM, Erdmann I, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled study. PNAS 2008;105:17908-17912, with permission from the National Academy of Sciences, USA.

Disclosure
The author states no conflicts of interest.

References

1. Moffitt JE, Golden DB, Reisman RE, Lee R, Nicklas R, Freeman T et al. Stinging insect hypersensitivity: A practice parameter update. J Allergy Clin Immunol 2004;**114**:869-886.
2. Lu G, Kochumian L, King TP. Sequence identity and antigenic cross-reactivity of white faced hornet allergen, also hyaluronidase, with other proteins. J Biol Chem 1995;**270**:4457-4465.
3. Hemmer W, Focke M, Kolarich D, Dalik I, Götz M, Jarisch R. Identfication by immunoblot of venom glycoproteins displaying IgE-binding N-glycans as crossreactive carbohydrate allergens in honey bee and yellow jacket venom. Clin Exp Allergy 2004;**34**:460-469.
4. Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and Vespula venom by estimation of sIgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy 2009;**64**:543-548.
5. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;**60**:1459-1470.
6. Goldberg A, Confino-Cohen R. Maintenance venom immunotherapy administered at 3-months intervals is both safe and efficacious. J Allergy Clin Immunol 2001;**107**:902-906.
7. Goldberg A, Confino-Cohen R. Effectiveness of maintenance bee venom immunotherapy administered at 6 months intervals. Ann Allergy Asthma Immunol 2007;**99**:352-357.
8. Müller UR. Bee venom allergy in beekeepers and their family members. Curr Opin Allergy Clin Immunol 2005;**5**:343-347.
9. Müller U, Rabson AR, Bischof M, Lomnitzer R, Dreborg S, Lanner A. A double-blind study comparing monomethoxy polyethylene glycol-modified honeybee venom and unmodified honeybee venom for immunotherapy. J Allergy Clin Immunol 1987;**80**:252-261.
10. Müller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. J Allergy Clin Immunol 1998;**101**:747-754.
11. Karamloo F, Schmid-Grendelmeier P, Kussebi F, Akdis M, Salagianni M, von Beust BR et al. Prevention of allergy by a recombinant multi-allergen vaccine with reduced IgE binding and preseved T cell epitopes. Eur J Immunol 2005;**35**:3268-3276.
12. Severino MG, Cortellini G, Bonadonna P, Francescato E, Panzini I, Macchia D et al. Sublingual immunotherapy for large local reactions caused by honey bee stings. J Allergy Clin Immunol 2008;**122**:44-88.
13. Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled study. PNAS 2008;**105**:17908-17912.
14. Martínez-Gómez JM, Johansen P, Erdmann I, Senti G, Crameri R, Kündig TM. Intralymphatic injections as a new administration route for allergen-specific immunotherapy. Int Arch Allergy Immunol 2009;**150**:59-65.

PART THREE

Mechanisms of allergy

MECHANISMS OF EARLY AND LONG TERM PROTECTION PROVIDED BY VENOM IMMUNOTHERAPY (VIT)

Cezmi A. Akdis

Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland

Venom immunotherapy (VIT) provides the only efficient protection against anaphylaxis in individuals with a high risk of a systemic reaction to an insect sting. VIT can restore normal immunity against allergens and provide patients with a lifetime tolerance to venom. Distinct mechanisms, involving specific cell populations, play a role in the different phases of allergen-specific immunotherapy (allergen-SIT) and are already activated after the initial exposure to the antigen (Fig. 1). T regulatory (Treg) cells are known to be involved in preventing sensitisation to allergens which has raised the question of whether therapies that target Treg cells could improve the efficacy of VIT.

Allergen-specific T cell response in non-allergic healthy individuals

Studies of the T cell response to allergens in healthy individuals have demonstrated responses that range widely from no detectable response to the involvement of active processes of systemic tolerance mediated by different subsets of Treg cells. In cultures of peripheral blood mononuclear cell (PBMC) from the majority of healthy individuals, T cells do not show any proliferative response to allergens, which is possibly due to the presence of a low number of specific T cells following a lack of exposure to the allergen. If a detectable allergen-specific T cell response does occur, in cultures from a healthy non-allergic individual, there is suppression of the response by Tr1 or CD4+FoxP3+ Treg cells (1-4).

The importance of Foxp3 in suppression of allergy was highlighted by the discovery of the X-linked syndrome of immune dysregulation, polyendocrinopathy and enteropathy (IPEX) (5, 6). IPEX patients spontaneously develop various allergic and autoimmune phenotypes and their CD4+CD25+ Treg cells are non-functional, indicating the existence of a population of dedicated suppressor cells. One patient with clinical manifestations of IPEX but normal FOXP3 gene function has been described. This patient had CD25 deficiency due to autosomal recessive mutations in this gene, thus CD25 deficiency results in an IPEX-like syndrome and suggests that FOXP3 is not required for normal IL-10 expression by human CD4 lymphocytes, whereas CD25 expression is (7). The relevance of the immune suppressive function of CD4+CD25+FOXP3+ Treg cells in human allergies has been investigated. Impaired skin infiltration of CD4+CD25+FOXP3+ Treg cells was observed in acute atopic dermatitis lesions (8) and FOXP3 mRNA expression was significantly increased in asthmatic patients receiving glucocorticoid treatment (9). Similarly, in patients with chronic rhinosinusitis with nasal polyps, decreased FOXP3 expression and a downregulation of TGF-b1 was observed when compared with controls and

chronic rhinosinusitis patients without nasal polyps (10). Consistent with these human observations, in a murine model of chronic asthma, there is accumulation of FoxP3+ Treg cells in local draining lymph nodes of the lung that correlates with spontaneous resolution of the condition (11).

In atopic dermatitis, CD4+CD25hiFoxP3+ Treg cells expressing cutaneous lymphocyte-associated antigen were increased, and associated with increased disease severity (12). Two subtypes of CD25hi Treg cells were identified on the basis of differential expression of the chemokine receptor CCR6. Despite a regulatory phenotype, activated CD25hi T cells that lack expression of CCR6 were found to promote Th2 responses (12) and increased during pollen season in allergic children (13). However, whether these CD4+CD25hi Treg cells directly contribute to inflammation, or their increased levels keep the inflammation at low levels remains unclear. Levels of circulating allergen-specific CD4+CD25hiFoxp3+ Treg cells do not show a major difference between non-atopic and atopic individuals (14). It was demonstrated that FOXP3 expression shows a negative correlation with IgE, eosinophilia and IFN-g levels, and FOXP3+/CD4+ ratio is significantly lowered in asthma and atopic dermatitis (15). Other studies have reported that numbers of CD4+CD25+ Treg cells are higher in non-allergic individuals (16, 17).

In non-allergic, healthy beekeepers, the antigen-specific peripheral T cell tolerance is modified during the beekeeping season. Th1 and Th2 cells show clonality and switch toward IL-10-secreting Tr1 cells, resulting in diminished T cell-related cutaneous late-phase responses in parallel with suppressed allergen-specific T cell proliferation and Th1 and Th2 cytokine secretion (4). This regulation is present as long as venom exposure persists and returns to initial levels two to three months after stings occur. In the same model, the upregulation of histamine receptor (HR) 2 on specific Th2 cells suppresses allergen-stimulated T cells and increases IL-10 production (4).

Using allergen-specific CD4+ Treg cells that resemble Th1-, Th2- and Tr1-like cells and are IFN-g-, IL-4- and IL-10-secreting respectively, it was shown that both healthy and allergic individuals exhibit all three subsets but in different proportions. In healthy individuals who show detectable IgG antibodies against allergens, Tr1 cells represent the dominant subset for common environmental allergens and utilise IL-10, TGF-beta, CTLA-4 and PD1 as suppressor molecules (2, 4). In contrast, allergic individuals have high numbers of allergen-specific IL-4-secreting T cells. Thus, the relative proportions of effector Th2 cells or Tr1 cells are decisive in the development of allergy or a healthy immune response (2).

Human CD4+Th1 cells predominantly express HR1, whereas CD4+Th2 cells express HR2, which results in differential histamine signalling (18). Histamine induces the production of IL-10 by dendritic cells (19), the production of IL-10 by Th2 cells (4, 20), and enhances the suppressive activity of TGF-beta on T cells (21). All of these effects are mediated by HR2, which is relatively highly expressed on Th2 cells and additionally mediates suppression of IL-4 and IL-13 production and T cell proliferation (4, 18). These findings suggest that HR2 may play a role in peripheral tolerance to allergens.

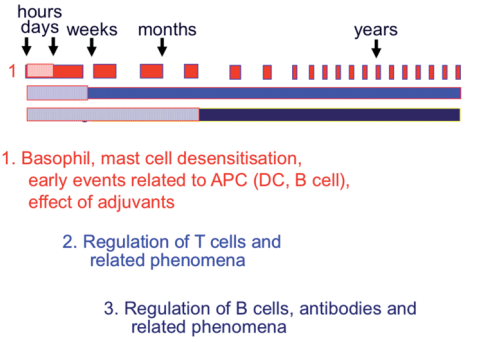


Fig. 1. Mechanisms of allergen-SIT involving different cell types are activated from the first exposure to antigen.

Immune response during allergen-specific immunotherapy

T-cell responses: The induction of a tolerant state in peripheral T cells represents an essential step in allergen-SIT. Peripheral T cell tolerance is characterised mainly by generation of allergen-specific Treg cells with suppressed proliferative and cytokine responses against the major allergen (22). This is initiated by autocrine action of IL-10 and TGF-beta, which are increasingly produced by the antigen-specific Tr1 cells (1, 23, 24) and these effects can be partially blocked by neutralising antibodies against secreted or membrane-bound IL-10 and TGF-beta. These cells express CD4 and CD25, which raises the question as to whether these are inducible Tr1 cells with upregulated CD25, or naturally occurring CD4+CD25+ Treg cells that produce suppressive cytokines (25). There is some evidence in adults of overlapping populations of circulating CD4+CD25+ Treg cells and IL-10- and TGF-beta-secreting Tr1 cells and it has been shown that CD4+CD25+ Treg cells from atopic donors have a reduced capability to suppress the proliferation of CD4+CD25- Treg cells following allergen-SIT (1, 3).

It has been suggested that upregulation of CD4+CD25+ Treg cells may play an important role in allergen-SIT. Recently, it was shown that grass pollen immunotherapy increased the expression of mucosal and peripheral T cell IL-10 and TGF-beta (26, 27). Increased numbers of CD25+FOXP3+ Treg cells in the nasal mucosa after immunotherapy, suggests that Treg cells play a role in the induction of allergen-specific tolerance in humans (28). Moreover, their increased numbers correlated with clinical efficacy and suppression of seasonal allergic inflammation (28).

The findings from studies of sublingual-SIT (SLIT) are generally similar to injection-SIT although, a few studies failed to detect any immunological changes (29, 30). In a recent study, higher numbers of circulating CD4+CD25+ Treg cells with increased expression of FoxP3 and IL-10 and reduced expression of IL-4 and IFN-g mRNA were detected after 4 weeks of sublingual-SIT (31). Proliferation was markedly reduced, but increased significantly after depletion of CD25+ cells or addition of anti-IL-10 antibodies. Neither TGF-beta levels nor cell-to-cell contact-mediated suppression of CD4+CD25+ Treg cells changed during the course of SLIT. Improvements in current understanding of the mechanisms of allergen-SIT, particularly the role of Treg cells in peripheral tolerance, open up possibilities for novel treatment strategies.

Immunoglobulin responses: The allergen-specific antibody response in non-allergic individuals ranges from undetectable levels of antibodies to relatively high amounts of specific IgG4, IgG1 and IgA in the absence or presence of low amounts of specific IgE (sIgE) (1, 32). The lack of efficient antigen presentation (due to exposure in small doses) may be responsible for an absence of response. Low exposure due to lack of accessibility and/or brief persistence of the allergens in secondary lymphoid organs has been demonstrated in mouse models (33); however, in humans, a single bee sting can induce IgE and IgG, and very small doses of T cell epitope peptides injected into skin can initiate allergic reactions (34-36). A single honeybee sting injects approximately 50-140 µg of venom containing 5-15 µg of the major allergen phospholipase A2 and is sufficient to induce IgE or IgG production against phospholipase A2 (36). The protein content of wasp venom is 10-20 times lower than that of honeybee venom (1.7-3.1 µg), nevertheless, a single sting is sufficient to induce IgE or IgG type antibodies against allergic components that are less than 10% of the protein content (36). Thus, 0.1-0.3 µg of allergen directly injected into the skin is sufficient to initiate an antibody response. However, it should be noted here that the immune response does not only depend on the allergen dose. Other molecules present in the venom, such as hyaluronidase, may exert adjuvant effects and affect the diffusion of the venom (37).

Interestingly, non-allergic beekeepers have a ratio of IgG4 to IgE that is approximately 1000 times higher than allergic individuals (38). In beekeepers, bee venom-specific IgG4 correlates with the number of annual stings and to the number of years spent in beekeeping. High exposure appears to induce clinical tolerance; 45% of beekeepers who were stung less than 25 times a year had a history of systemic allergic reactions to bee venom, whereas those with more than 200 stings per year did not have any allergy (36).

IgG4 has unique structural and functional features compared to other IgGs. Its heavy chains may exchange in a way that leads to functional monomeric antibodies, it has low affinity for certain Fcg receptors, and it does not activate complement (39). IgG4 antibodies exchange Fab arms by swapping a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule, resulting in bispecific antibodies that are capable of inhibiting immune complex formation by other isotypes (40). Thus, they may play an anti-inflammatory role in immunity.

Although peripheral tolerance has been demonstrated in specific T cells, the ability of B cells to produce sIgE antibodies is not eliminated during allergen-SIT (22). Allergen-SIT frequently induces a transient increase in serum specific IgE, followed by gradual decrease over months or years of treatment (41, 42) and prevents elevation of the serum sIgE during the pollen season in sensitive patients (43). Serum levels of sIgE and IgG4 antibodies increase during the early phase of allergen-SIT. The increase in antigen-specific IgG4 is more striking and therefore the ratio of sIgE to IgG4 significantly decreases between 6 months and three years of treatment. A similar change in specific isotype ratio has been observed in SIT of various allergies (22, 26). Moreover, IL-10 produced and progressively secreted during allergen-SIT, appears to counter-regulate synthesis of antigen-specific IgE and IgG4 antibodies. IL-10 potently suppresses both total and allergen-specific IgE, and simultaneously increases IgG4 production (23, 44). Therefore, IL-10 not only generates T cell tolerance, it also regulates specific isotype formation and skews the sIgE response towards an IgG4 dominated phenotype.

Direct influence of Tr1 and Foxp3+ Treg cells on B cells and the induction of IgG4 and suppression of IgE has been demonstrated in cell cultures of peripheral blood cells of healthy individuals (45). In a mouse model of food allergy, antigen-specific secretory IgA antibodies in the gut were decreased, suggesting a role for secretory IgA in peripheral tolerance to foods. Peyer's patch CD31 cells were primarily involved and favoured IgA production through the release of IL-10 and TGF-beta. Low production of IL-10 in Peyer's patches favoured the symptoms of food allergy (46). There is increasing evidence that allergen-SIT also influences the blocking activity of IgG4 on IgE-mediated responses. A cellular assay that detects allergen-IgE binding using flow cytometry, has been used to detect 'functional' specific immunotherapy-induced changes in IgG antibody activity. Results suggest that successful specific immunotherapy is associated with an increase in IgG blocking activity that is not solely dependent on the quantity of IgG antibodies (47). Thus, it seems to be relevant to measure the blocking activity of allergen-specific IgG or IgG subsets, particularly IgG4 and also IgG1 rather than crude IgG levels in sera.

In the context of these findings, the safety and efficacy of anti-IgE treatment in the induction phase of allergen-SIT needs to be reviewed. Anti-IgE mAb pretreatment enhances the safety of SIT for allergic rhinitis and may be an effective strategy to permit more rapid and higher doses of allergen immunotherapy (48, 49). However, its effects on the long-term efficacy of SIT are still under investigation.

Disclosure
The author states no conflicts of interest.

References

1. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003;**33**:1205-1214.
2. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 2004;**199**:1567-1575.
3. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet 2004;**363**:608-615.
4. Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. J Exp Med 2008;**205**:2887-2898.
5. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest 2000;**106**:R75-81.
6. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buis tN, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat. Genet. 2001;**27**:18-20.
7. Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. J Allergy Clin Immunol 2007;**119**:482-487.
8. Verhagen J, Akdis M, Traidl-Hoffmann C, Schmid-Grendelmeier P, Hijnen D, Knol EF, et al. Absence of T-regulatory cell expression and function in atopic dermatitis skin. J Allergy Clin Immunol 2006;**117**:176-183.
9. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, et al. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol 2004;**114**:1425-1433.
10. Van Bruaene N, Perez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N, et al. T-cell regulation in chronic paranasal sinus disease. J Allergy Clin Immunol 2008;**121**:1435-1441, 1441 e1431-1433.
11. Carson WFt, Guernsey LA, Singh A, Vella AT, Schramm CM, Thrall RS. Accumulation of regulatory T cells in local draining lymph nodes of the lung correlates with spontaneous resolution of chronic asthma in a murine model. Int Arch Allergy Immunol 2008;**145**:231-243.
12. Reefer AJ, Satinover SM, Solga MD, Lannigan JA, Nguyen JT, Wilson BB, et al. Analysis of CD25hiCD4+ "regulatory" T-cell subtypes in atopic dermatitis reveals a novel T(H)2-like population. J Allergy Clin Immunol 2008;**121**:415-422 e413.
13. Jartti T, Burmeister KA, Seroogy CM, Jennens-Clough ML, Tisler CJ, Salazar LP, et al. Association between CD4(+)CD25(high) T cells and atopy in children. J Allergy Clin Immunol 2007;**120**:177-183.
14. Maggi L, Santarlasci V, Liotta F, Frosali F, Angeli R, Cosmi L, et al. Demonstration of circulating allergen-specific CD4+CD25highFoxp3+ T-regulatory cells in both nonatopic and atopic individuals. J Allergy Clin Immunol 2007;**120**:429-436.
15. Orihara K, Narita M, Tobe T, Akasawa A, Ohya Y, Matsumoto K, et al. Circulating Foxp3+CD4+ cell numbers in atopic patients and healthy control subjects. J Allergy Clin Immunol 2007;**120**:960-962.
16. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med 2004;**199**:1679-1688.
17. Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pita O, Girolomoni G. Human CD25+ regulatory T cells maintain im-

mune tolerance to nickel in healthy, non-allergic individuals. J Immunol 2003;**171**:5760-5768.
18. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OAR, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 2001;**413**:420-425.
19. Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. J. Clin. Invest. 2001;**108**:1865-1873.
20. Osna N, Elliott K, Khan MM. Regulation of interleukin-10 secretion by histamine in TH2 cells and splenocytes. Int. Immunopharmacol. 2001;**1**:85-96.
21. Kunzmann S, Mantel P-Y, Wohlfahrt JG, Akdis M, Blaser K, Schmidt-Weber CB. Histamine enhances TGF-beta1-mediated suppression of Th2 responses. Faseb J. 2003;**17**:1089-1095.
22. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Muller U, et al. Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. J Clin Invest 1996;**98**:1676-1683.
23. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998;**102**:98-106.
24. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. J Allergy Clin Immunol 2003;**111**:1255-1261.
25. Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol 2005;**116**:961-968; quiz 969.
26. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 2004;**172**:3252-3259.
27. Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. J Immunol 2007;**178**:4658-4666.
28. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. J Allergy Clin Immunol 2008;**121**:1467-1472, 1472 e1461.
29. Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. Allergy 2006;**61**:151-165.
30. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005;**60**:4-12.
31. Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. J Allergy Clin Immunol 2007;**120**:707-713.
32. Pereira EA, Silva DA, Cunha-Junior JP, Almeida KC, Alves R, Sung SJ, et al. IgE, IgG1, and IgG4 antibody responses to Blomia tropicalis in atopic patients. Allergy 2005;**60**:401-406.
33. Zinkernagel RM, Ehl S, Aichele P, Oehen S, Kundig T, Hengartner H. Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. Immunol Rev 1997;**156**:199-209.
34. Müller UR, Mosbech H. Position paper: Immunotherapy with hymenoptera venoms. Allergy 1993;**48**:36-46.
35. Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. J. Exp. Med. 1999;**189**:1885-1894.
36. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy 2005;**60**:1339-1349.
37. Girish KS, Kemparaju K. Inhibition of Naja naja venom hyaluronidase: role in the management of poisonous bite. Life Sci 2006;**78**:1433-1440.
38. Carballido JM, Carballido-Perrig N, Kägi

MK, Meloen RH, Wüthrich B, Heusser CH, et al. T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A2. J.Immunol. 1993;**150**:3582-3591.

39. Aalberse RC, Schuurman J. IgG4 breaking the rules. Immunology 2002;**105**:9-19.
40. Van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 2007;**317**:1554-1557.
41. Van Ree R, Van Leeuwen WA, Dieges PH, Van Wijk RG, De Jong N, Brewczyski PZ, et al. Measurement of IgE antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. Clin Exp Allergy 1997;**27**:68-74.
42. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. J Allergy Clin Immunol 1982;**70**:261-271.
43. Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. J Allergy Clin Immunol 1988;**82**:439-446.
44. Punnonen J, De Waal Malefyt R, Van Vlasselaer P, Gauchat J-F, De Vries JE. IL-10 and viral IL-10 prevent IL-4-indiced IgE synthesis by inhibiting the accessory cell function of monocytes. J. Immunol. 1993;**151**:1280-1289.
45. Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. Allergy 2008;**63**:1455-1463.
46. Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. J Allergy Clin Immunol 2004;**114**:377-382.
47. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol 2004;**4**:313-318.
48. Casale TB, Busse WW, Kline JN, Ballas ZK, Moss MH, Townley RG, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2006;**117**:134-140.
49. Klunker S, Saggar LR, Seyfert-Margolis V, Asare AL, Casale TB, Durham SR, et al. Combination treatment with omalizumab and rush immunotherapy for ragweed-induced allergic rhinitis: Inhibition of IgE-facilitated allergen binding. J Allergy Clin Immunol 2007;**120**:688-695.

PART FOUR

Practical sessions

IN VITRO TESTS

Wolfgang Hemmer[1] *and Gunter Sturm*[2]

[1]Floridsdorfer Allergiezentrum, Vienna, Austria
and [2]Department of Dermatology Medical University of Graz, Graz, Austria

In vitro measurement of venom-specific IgE (sIgE) is a conventional test that is usually used in addition to skin testing, and, in some centers, may even be used as the primary diagnostic approach. The presence of IgE antibodies may be demonstrated directly, by measuring circulating IgE in the serum, or indirectly, by cellular tests that either measure the release of IgE-dependent mediator from blood cells or allergen-specific up-regulation of surface markers on sensitised basophils.

Measurement of venom-specific IgE

Measurement of venom-sIgE) is routinely carried out by fully-automated commercially available systems, often collectively termed RAST (radio-allergo-sorbent test). Allergens are coupled to a (semi)-solid phase and bound serum IgE is detected by fluorescent/chemiluminescent enzyme- or formerly radio-labeled anti-IgE (e.g. ImmunoCAP®, Immulite 2000®, HYTEC®). Alternatively, sIgE is bound by anti-IgE on the surface of a test well, and then incubated with labelled allergens (e.g. ADVIA Centaur®). Tests using whole venoms are available for most relevant Hymenoptera, including honeybee, *Vespula* (usually a mix of European and American species), *Dolichovespula* (only American species available), European hornet (*Vespa*), paper wasp (for European patients *Polistes dominulus* should be used instead of American mix), bumblebee (*Bombus terrestris*) and fire ant (*Solenopsis*, whole-body extract). Tests based on single recombinant venom allergens are currently coming onto the market. Some test systems also offer the possibility to measure allergen-specific IgG/IgG4 antibodies.

The specificity of sIgE measurements continues to be debated: depending on the methods and venoms used, the specificity of sIgE detection was found to range between 60% and 94% (1, 2). Manufacturers' claims for sensitivity and specificity should be viewed with caution as these are frequently measured under optimal conditions and control subjects with high tIgE levels, positive skin tests, and atopic disposition are generally ruled out in order to obtain optimum specificities.

In routine practice, co-sensitisation to multiple allergens, due to *in vitro* cross-reactions, makes it difficult for the clinician to make an unequivocal diagnosis. In Hymenoptera allergy, cross-reactions due to CCDs that result in double-positive test results for honeybee and yellow jacket venom have been frequently described (3, 4). The frequency of double-positive test results ranges from 10% (Phadebas® RAST) to 30% (ImmunoCAP®) (5). Some double-positive results may be explained by clinically irrelevant cross-reactivity *in vitro*: during the IgE assay, sIgE antibodies are free and can easily bind to all available epitopes. This binding is monova-

lent and each IgE molecule bound to one genuine or cross-reacting epitope generates a signal in the assay well. This is not the case *in vivo*, where the allergic response requires efficient cross-linking of at least two high-affinity IgE receptors on basophils or mast cells (bivalent interaction) in order to induce cell activation and mediator release.

Western blotting is another technique that enables the simultaneous determination of IgE specificities for different venom components. Proteins loaded on a polyacrylamide gel are separated by electrophoresis (SDS-PAGE) according to their molecular weight and subsequently blotted to nitrocellulose or polyvinylidene fluoride membranes. After incubation of single membrane strips with patients' sera, bound IgE is detected by enzyme- or radio-labelled anti-IgE. Ready-to-use test strips with pre-separated allergens were commercially available until recently but have been discontinued (AlaBLOT®).

IgE inhibition test

Inhibition experiments are useful in identifying the primary venom in patients with reactivity to two or more venoms where the identity of the stinging insect is unknown. Inhibition experiments have been successfully used to resolve diagnostic problems, such as honeybee-*Vespula*, *Vespula-Polistes*, and *Vespula-Vespa* double-positivity (4, 6-9). The IgE inhibition technique is relatively simple and can be carried out routinely in clinical practice. The classical reciprocal IgE inhibition technique employed the respective venoms as inhibitors, however, it was subsequently demonstrated that inclusion of a suitable plant glycoprotein (e.g. bromelain, or grass pollen extracts), as an inhibitor of cross-reactive carbohydrate determinants (CCD), could enable discrimination between protein- and carbohydrate-based cross-reactivity (3, 6). Due to the higher amounts of CCD in honeybee venom as compared to vespid venoms, *Vespula* venom may be a poor inhibitor of honeybee CCD in sera with high anti-CCD IgE levels, thus potentially leading to over-diagnosis of true double sensitisation.

A sample protocol for a reciprocal IgE inhibition test in a honeybee-*Vespula* double-positive patient is shown below:

1. Four aliquots of the patient's serum are mixed with equal volumes of bee venom, *Vespula* venom, CCD inhibitor, and buffer (negative control) respectively and incubated for 2-4 h at room temperature or overnight at 4°C. Increasing concentrations of inhibitor proteins may be used to obtain dose-response curves, however, a single final inhibitor concentration of approx. 100 µg/ml is sufficient for routine work (3, 6-8). This may be achieved by mixing e.g. 100 µl serum with 50 µl of a venom stock solution with a protein concentration of 300 µg/ml. In case of very high sIgE levels, pre-dilution of serum may be required.

2. After incubation, sIgE measurement is performed on all venom samples (8 tests in total). By comparing the score from the non-inhibited control sample with those from the inhibited samples, the degree of inhibition may be calculated for each inhibitor using the formula [100 - (score inhibited sample / score negative control) * 100]. There is currently no general consensus on the definition of what defines full and partial cross-inhibition. According to Straumann *et al.* (6), full cross-reactivity occurs if inhibition by the heterologous venom reaches at least 85% of the inhibition achieved with the homologous venom (autoinhibition). Inhibition values below 20% are usually considered to be non-specific.

While sIgE inhibition has the potential to discriminate between cross-reactivity and true double sensitisation, and often enables identification of the primary venom, results in everyday practice are not always unequivocal and conclusive, and inhibition in sera with very low levels of venom-sIgE is often unreliable. While sIgE inhibition involves additional workload and expenses, some of the cost may be recovered by avoiding unnecessary double immunotherapy (6).

IgE inhibition test - case reports

Here we present two cases demonstrating the use of sIgE inhibition in patients with multiple positive venom-sIgE results obtained by ImmunoCAP®:

Case 1 (Fig. 1): Patient had a systemic reaction after sting from unknown insect. ImmunoCAP® results were: double-positive to honeybee (5.2 kU/L) and *Vespula* (18.8 kU/L), bromelain (CCD) 5.6 kU/L.

Interpretation: Primary *Vespula* venom allergy. IgE-binding to *Vespula* venom is only inhibited by *Vespula* itself. IgE reactivity with honeybee venom is entirely due to CCD and thus may be considered clinically irrelevant. Failure to include a CCD inhibitor would have led to the wrong diagnosis of true double-sensitisation, indicating VIT with two venoms.

Case 2 (Fig. 2): Patient had a systemic reaction after a sting by a European hornet. Routine ImmunoCAP® results were: *Vespula* 12.6 kU/L, *Vespa* 12.5 kU/L, honeybee 5.6 kU/L, bromelain (CCD) 0.61 kU/L.

Interpretation: IgE-binding to *Vespa* venom is inhibited equally by *Vespa* and *Vespula* venom whereas binding to *Vespula* venom is not inhibited completely by *Vespa* venom. This indicates a primary sensitisation to *Vespula* venom and cross-reactivity with *Vespa* venom. In addition, protein-specific cross-sensitisation to honeybee venom is present (it is currently not known whether this was due to the high sequence identity between hyaluronidases or other cross-reactive allergens). CCD do not play a significant role in cross-reactivity despite a slightly positive bromelain sIgE result.

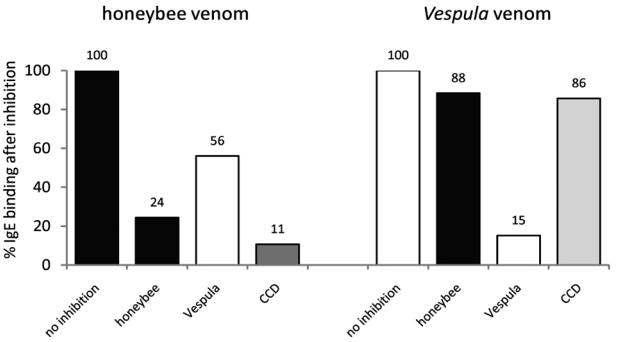


Fig. 1. IgE inhibition test in a double-positive patient with an allergic reaction after a sting from an unknown insect. Bars represent percent IgE binding to honeybee venom (left) and *Vespula* venom (right), respectively, after inhibition with different inhibitors in relation to the non-inhibited serum.

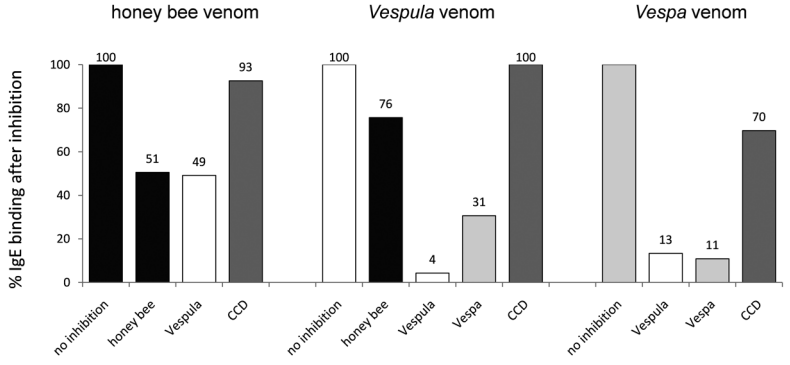


Fig. 2. Identification of the primary venom in a patient with an allergic reaction after a European hornet (*Vespa crabro*) sting by IgE inhibition test. Bars represent percent IgE-binding to honeybee venom (left), *Vespula* venom (middle), and *Vespa* venom (right), respectively, after inhibition with different inhibitors in relation to the non-inhibited serum.

Basophil activation test (BAT) and clinical relevance of venom-specific IgE

Compared with serum IgE detection, cellular tests like the basophil activation test (BAT) offer the advantage of being able to demonstrate functional responses, as positive test results only occur after successful cross-linking. Currently, BAT with CD63 is the best clinically-validated test, while BAT based on CD203c still requires further development. In contrast to the BAT, which is now generally accepted as an additional and reliable diagnostic tool in Hymenoptera allergy, other *in vitro* tests, such as histamine and leukotriene release tests have practical and technical shortcomings and therefore restricted clinical application.

A common problem with the current diagnostic tools is that, generally, test results do not correlate with the severity of the sting reaction and no test provides information on the clinical relevance of the sensitisation, making it impossible to predict future reactions and their severity (10). Recently, we reported that asymptomatic sensitisation in subjects without a previous sting reaction depended on the levels of total IgE (tIgE). Up to 66.7% of subjects with elevated tIgE had detectable sIgE to Hymenoptera venom (Fig. 3) (11).

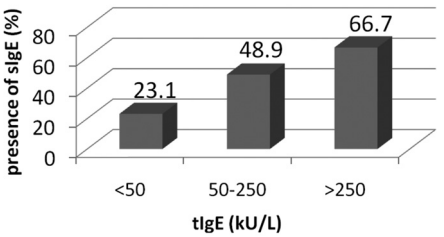


Fig. 3. The frequency of asymptomatic sensitisation to honeybee (i1) and/or vespid venom (i3) is dependent on the total serum IgE level.

As it remained unclear whether these sensitisations were relevant, we performed sting challenges in 94 asymptomatically sensitised subjects in a follow-up study: only 6 (4.6%) systemic sting reactions occurred in 131 sting challenges, which confirmed our hypothesis that most of these sensitisations are not clinically relevant. In a comparison of the different diagnostic tools, the BAT correlated best with the outcome of sting challenges. Comparing three different methods to detect serum IgE (ImmunoCAP®, Immulite 2000®, and ADVIA Centaur®), BAT gave the lowest frequency of double-positive results and correlated best with patient's history (Fig. 4). As the ADVIA system has recently been withdrawn from the market, the BAT remains as only *in vitro* method, which is able to discriminate between asymptomatic and clinically relevant sensitisation. BAT is now generally accepted as a useful additional tool in the diagnosis of Hymenoptera allergy. However, whether the BAT is able to monitor the efficacy of Hymenoptera venom immunotherapy and whether it is helpful in patients with negative skin tests and negative sIgE requires further investigation.

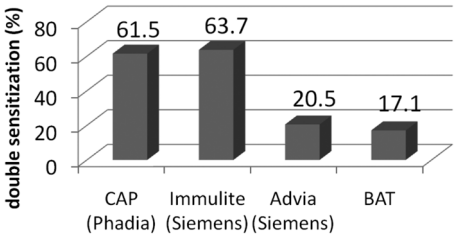


Fig. 4. Frequency of double sensitisation to honeybee (i1) and vespid venom (i3) in different sIgE detection systems.

Disclosure

The authors state no conflicts of interest.

References

1. Schäfer T, Przybilla B. IgE antibodies to Hymenoptera venoms in the serum are common in the general population and are related to indications of atopy. Allergy 1996;**51**:372-377.
2. Sturm GJ, Böhm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. Allergy 2004;**59**:1110-1117.
3. Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhrl S, et al. Antibody binding to venom carbohydrates is a fre-

quent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol 2001;**108**:1045-1052.
4. Hemmer W, Focke M, Kolarich D, Dalik I, Götz M, Jarisch R. Identification by immunoblot of venom glycoproteins displaying immunoglobulin E-binding N-glycans as cross-reactive allergens in honeybee and yellow jacket venom. Clin Exp Allergy 2004;**34**:460-469.
5. Egner W, Ward C, Brown DL, Ewan PW. The frequency and clinical significance of specific IgE to both wasp (Vespula) and honey-bee (Apis) venoms in the same patient. Clin Exp Allergy 1998;**28**:26-34.
6. Straumann F, Bucher C, Wüthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Int Arch Allergy Immunol 2000;**123**:268-274.
7. Kosnik M, Korosec P, Silar M, Music E, Erzen R. Wasp venom is appropriate for immunotherapy of patients with allergic reactions to the European hornet sting. Croat Med J 2002;**43**:25-27.
8. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hübsch-Müller C, Enk A. In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. Allergy 2006;**61**:1220-1229.
9. Severino MG, Campi P, Macchia D, Manfredi M, Turillazzi S, Spadolini I, et al. European Polistes venom allerg. Allergy 2006;**61**:860-863.
10. van der Linden PW, Hack CE, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 324 subjects with a previous anaphylactic reaction: current criteria for insect-venom hypersensitivity do not predict the occurrence and the severity of anaphylaxis. J Allergy Clin Immunol 1994;**94**:151-159.
11. Sturm GJ, Schuster C, Kranzelbinder B, Wiednig M, Groselj-Strele A, Aberer W. Asymptomatic Sensitization to Hymenoptera Venom Is Related to Total Immunoglobulin E Levels. Int Arch Allergy Immunol 2008;**148**:261-4.

PRESENTATION OF SOME DIFFICULT CASES IN DIAGNOSIS

Ulrich Müller[1] and Paolo Campi[2]

[1]Allergiestation Medizinische, Klinik Ziegler Spital, Bern, Switzerland
and [2]Allergy Clinic, Nuovo Ospedale San Giovanni di Dio Florence, Florence, Italy

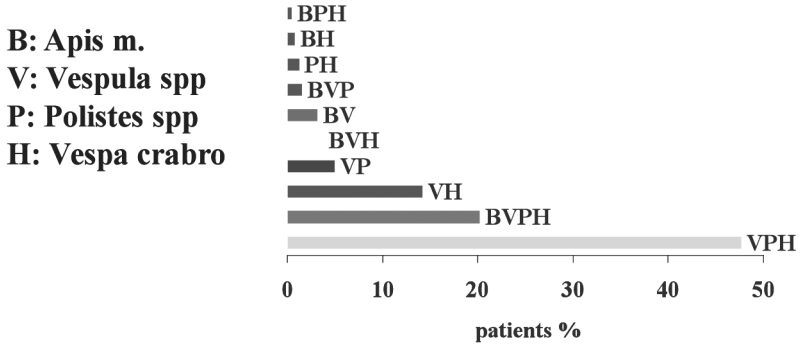
Choice of venom for immunotherapy

One of the most difficult tasks in treating Hymenoptera venom allergy is the choice of venom for immunotherapy. Figures from the San Giovanni di Dio hospital in Florence indicate that in 59% of cases the patient is not able to identify the stinging insect. The time of year and the events surrounding the sting can sometimes give clues to the identity of the insect. Stings that occur while eating outside are usually caused by *Vespula* while stings on the feet while walking barefoot are more likely to be caused by bees. The presence of the stinger in the skin indicates the stinging insect was a bee, and in some cases the size of the insect may give a clue to its identity, as is the case with *Vespa crabro* (1, 2). To compound the problem of identification, our figures show that in 59% of venom allergy cases, skin tests were positive for more than one venom. Therefore, if the physician is unable to identify the stinging insect the patient must be treated with all the venoms that give a positive skin test.

The most frequent multiple reactivity seen in patients at the San Giovanni di Dio hospital in Florence is to venoms of the Vespids: *Vespula spp.*, *Polistes dominulus*, and *Vespa crabro*. These insects cause the majority of stings in Italy and are responsible for multiple reactivity in around 50% of patients with multiple positive skin tests (Fig. 1). Before starting immunotherapy, the physician must distinguish between multiple reactivity resulting from stings by different insects (multiple sensitisation), and sensitisation to one venom (the primary venom) with multiple reactivity caused by cross-reactivity (2). Cross-reactivity may be caused by individual cross-reacting allergens in both venoms, e.g. hyaluronidase (which has approximately 50% sequence identity) or cross-reacting carbohydrate determinants (CCDs).

The specific IgE (sIgE)-inhibition test is one method for diagnosis and choice of venom for immunotherapy in patients with multiple reactivity unable to reliably identify the stinging insect. sIgE-inhibition is performed by mixing the patient's serum with different concentrations of venom before performing the sIgE measurement (e.g., ImmunoCAP® test) (7). First, the homologous inhibition is tested to determine if the sIgE test is inhibited by previous adsorption with the same venom. Inhibition never reaches 100%, and figures higher than 90% are acceptable. Reciprocal inhibition is then tested to determine if the sIgE test is inhibited by previous adsorption with another venom and vice versa. In a study of 45 patients with non-discriminative skin/sIgE (ImmunoCAP®) positivity to both *Vespula* and *Polistes* venoms, Caruso and colleagues demonstrated that the venom of *Polistes dominulus* effectively bound *Vespula*-specific IgE in 56% of cases, and

Skin tests results in 1190 patients with multiple reactivity



S. Giovanni di Dio Hospital, Florence

Fig. 1: Percentage of multiple reactivity in skin tests results.

vice versa in 13% of cases (7). Thus, in 31 of the 45 patients, the multiple reactivity to venoms was the result of cross-reactions and the IgE-inhibition test excluded true double sensitisations. In cases that are not due to multiple sensitizations, and where a primary venom can be identified, primary-venom therapy should also protect the patient from exposure to cross-reactive venoms (3-7). Thus, in this study, 31 of the 45 subjects received immunotherapy with only one venom (7).
An alternative method to distinguish between true multiple sensitisation, notably double positivity, due to cross-reactivity between honeybee and *Vespula* venom, is by measuring the serum specific IgE to the species-specific, recombinant, non glycosylated major allergens Api m 1 and Ves v 5, as it was performed in a study (8). As double positivity of IgE to bee and *Vespula* venom is often caused by cross-reactions, especially to CCDs, the presence of serum IgE to both Api m 1 and Ves v 5 indicates true double sensitisation and immunotherapy with both venoms is required. With this method it was possible to reduce double positivity in 200 venom allergic patients - and thus the need to treat with both venoms - from over 50% to 17% (8).

Clinical cases - multiple positive skin tests

Here we present two cases of multiple positive skin tests in which the choice of the venom was made with the help of IgE-inhibition. In both cases the patient had a severe reaction to the sting of an unidentified insect and skin tests and serum specific IgE were positive for both *Vespula* and *Polistes dominulus* venom.

Case 1: In this case (Table 1), *Polistes dominulus* venom completely adsorbed specific IgE against venom of *Vespula spp*., indicating *Polistes dominulus* as the primary venom. The patient was treated with the primary venom only.
Case 2: In this case (Table 2), reciprocal inhibition between *Vespula spp.* and *Polistes dominulus* venom was only partial, indicating that the patient has sIgE for both venoms. The patient required treatment with both venoms.
The use of a sIgE-inhibition test increases the number of tests that must be performed, increasing the cost of the diagnosis. However, it avoids the cost of performing immunotherapy with two venoms.

Table 1: Case no.1, IgE-inhibition between *Vespula spp.* and *Polistes dominulus*

Inhibiting venom (µg/ml)	***Vespula* IgE inhibited by *Vespula* (% of inhibition)**	***Polistes* IgE inhibited by *Polistes* (% of inhibition)**	***Vespula* IgE inhibited by *Polistes* (% of inhibition)**	***Polistes* IgE inhibited by *Vespula* (% of inhibition)**
0	0	0	0	0
0,3	31	11	2	5
3	89	45	20	11
30	93	95	97	18
300	96	96	98	31

Result: *Vespula* IgE is completely adsorbed by *Polistes* venom (the "primary venom"). Treatment with only *Polistes* venom is indicated.

Table 2: Case no.2, IgE-inhibition between *Vespula spp.* and *Polistes dominulus*

Inhibiting venom (µg/ml)	***Vespula* IgE inhibited by *Vespula* (% of inhibition)**	***Polistes* IgE inhibited by *Polistes* (% of inhibition)**	***Vespula* IgE inhibited by *Polistes* (% of inhibition)**	***Polistes* IgE inhibited by *Vespula* (% of inhibition)**
0	0	0	0	0
0,3	28	15	5	8
3	87	51	18	15
30	94	88	30	18
300	97	94	40	30

Result: there is some cross-reactivity between the two venoms, but neither is able to completely adsorb the specific IgE against the other venom.
Treatment with both venoms is indicated.

Large local reactions

A large local reaction (LLR) is a swelling at the site of a sting that exceeds 10 cm in diameter and is accompanied with erythema and itching. LLRs can affect an entire extremity and frequently last for 3-5 days, sometimes for several weeks. Usually, LLRs are treated with oral corticosteroids. LLRs are more common than systemic reactions and occur in 5-26% of the population compared with 0.3-8.9% who experience systemic reactions. Among 730 cases of LLR studied, venom sensitisation was documented by diagnostic tests in 83% (9). In subjects who experience a LLR, the risk of a systemic reaction to a subsequent sting is about 5-10%, whereas in subjects who have no reaction to a sting, but who show a positive skin test, the risk of a subsequent systemic reaction is 17% (10). Although diagnostic tests are indicated in all patients with a history of a systemic reaction, the current clinical guidelines do not recommend them in subjects with a history of LLR, and VIT is not recommended for patients with LLR because of the low risk of a subsequent systemic reaction (2).

Nevertheless, many patients fear a systemic reaction to a subsequent sting and practitioners are frequently asked by patients to perform a skin test following a LLR. The question then arises of what to do if the test is positive. Some practitioners give patients injectable epinephrine to reduce the patient's fear of a systemic reaction to a sting. On the other hand LLRs may on their own cause significant morbidity. In some cases swelling of an extremity can last for more than a week and in frequently exposed subjects, such as gardeners and beekeepers, this can severely affect the patient's ability to work and quality of life. A sting challenge in 41 patients with previous LLR and positive skin tests, resulted in a LLR of 16 cm or larger in 34 patients (83%) (11). In the same publication it was shown in a placebo-controlled trial that VIT decreased the size and duration of LLR by 42% and 53% respectively after 7-11 weeks of treatment. Results were similar after one year and improved to 60% and 70%, respectively after two and four years and patient satisfaction with treatment was over 90% (11). Thus, SLIT may be a promising treatment of insect venom hypersensitivity in patients with LLR.

Disclosure

The authors state no conflicts of interest.

References

1. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Mueller U. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice Allergy 2005;**60**:1459-1470.
2. Bilò BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG. Diagnosis of hymenoptera venom allergy. Allergy 2005;**60**:1339-1349.
3. Hoffman DR. Allergens in Hymenoptera venom. XV. Immunologic basis of vespid venom cross reactivity. J Allergy Clin Immunol 1985;**75**:611-613.
4. Hamilton RG, Wisenauser JA, Golden DBK, Valentine MD, Adkinson F. Selection of Hymenoptera venoms for Immunotherapy on the basis of patient's IgE antibody cross-reactivity. J Allergy Clin Immunol 1993;**92**:651-659.
5. Reisman RE, Wypych JI and Lazell MI. Further studies with both honey-bee and yellow jacket venom specific IgE. Int Archs Allergy Immunol 1987;**82**:190-194.
6. Straumann F, Bucher C, Wuthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Int Arch Allergy Immunol 2000;**123**:268-274.
7. Caruso B, Bonadonna P, Severino MG, Manfredi M, Dama A, Schiappoli M et al. Evaluation of the IgE cross-reactions among vespid venoms. A possible approach for the choice of immunotherapy. Allergy 2007;**62**:561-564.
8. Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy 2009;**64**:543-548.
9. Bilò BM, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. Clin Exp Allergy 2009;**39**:1467-1476.
10. Golden DB, Marsh DG, Freidhoff LR, Kwiterovich KA, Addison B, Kagey-Sobotka A et al. Natural history of Hymenoptera venom sensitivity in adults. J Allergy Clin Immunol. 1997;**100**:760-766.
11. Golden DB, Kelly D, Hamilton RG, Craig TJ. Venom immunotherapy reduces large local reactions to insect stings. J Allergy Clin Immunol 2009 ;**123**:1371-1375.

UP-DOSING OF VENOM IMMUNOTHERAPY: WHICH SCHEDULE IS BEST?

M. Beatrice Bilò

Allergy Unit, Department of Internal Medicine, Allergy, Immunology and Respiratory Diseases, University Hospital, Ancona, Italy

Introduction

Many treatment protocols have been designed for the induction phase of venom immunotherapy (VIT) (1). These vary with respect to the number of injections, venom doses, and time needed to reach the final dose (Table 1). A conventional regimen involves increasing doses in weekly intervals for outpatients, in rush regimen the induction phase lasts 4-7 days for inpatients, in the ultrarush protocol the maintenance dose is reached within 1-2 days or in a few hours, and the cluster regimen is a modified rush approach schedule which involves giving several injections at 15 to 30 minute intervals during the first visit and reaches a maintenance dose in about six weeks. Rush and ultrarush protocols are particularly useful in highly exposed subjects (e.g. beekeepers) or in patients who are referred to the specialist just prior to the start of the insect season. In addition, the cost of administering ultrarush VIT is lower than that of the slower protocols. These fast protocols are currently used in most but not all European countries (2).

Starting and maintenance doses

The starting dose of VIT is between 0.001 and 0.1 µg (Table 1). However, in a recent paper the authors demonstrated that initiating VIT at the 1 µg dose can be applied safely in rush protocols, both in adults and in children (3). They performed a rush protocol in 62 inpatients, and a modified rush protocol in 670 outpatients, both showed no systemic reaction (SR) when starting with 1 µg of venom (3).

The recommended maintenance dose is 100 µg of venom protein, corresponding to one to two bee stings and to many more *Vespula* stings and affords better protection than a 50 µg dose (1). Higher maintenance doses (200 µg or more) are recommended for beekeepers, who may be stung by several insects at the same time and in treatment failures or incomplete treatment success. If two or more venoms are required, they should be administered in separate protocols a few days apart. The general consensus is that the maintenance interval should be kept at four weeks for the first year, extended to six weeks in the second year, and then to eight weeks if VIT is continued over five years, provided that the treatment is tolerated (1). A longer interval is not recommended for honeybee allergic patients since beekeepers with less than 10 stings a year are those who developed SRs most frequently. Moreover, studies assessing the possibility of extending the maintenance interval up to 12 weeks included a small a sample of patients, mainly with vespid allergy, efficacy was evaluated mainly by in-field stings (1).

Recently, two studies evaluated the safety

and efficacy of a further prolonged interval between injections of 6 months, with conflicting results that are mainly related to a different selection of patients (4, 5). Hopefully within the next few years multicentre clinical trials will yield results allowing us to select patients in which the interval between injections can be extended without risk. While the dose-up phase of VIT should be performed by an allergist, in some countries maintenance treatment is continued by general practitioners.

Table 1. Some examples of treatment protocols for VIT (original)

DAY	HOUR	CONVENTIONAL*	CLUSTER*	RUSH*	ULTRARUSH*
1	0	0.01	0.001	0.01	0.1
	0.5	0.1	0.01	0.1	1
	1		0.1	1	10
	1.5				20
	2.5			2	30
					40
2	0			4	
	1			8	
				10	
				20	
3	0			40	
	1			60	
	2			80	
4	0			100	
8	0	1	1	100	
	1	2	5		
			10		
15	0	4	20	100	50
	1	8	30		50
22	0	10	50		100
	1	20	50		
29		40	100	100	
36		60	100		
43		80		100	100
50		100			
57		100			
64			100		
71		100		100	100
85		100			
92			100		
99				100	100
106			100		

* Dose in µg venom
Further injections of the maintenance dose of 100 µg every four weeks during the first year, every six weeks in the second year, and then every eight weeks if VIT is continued over five years (1).

Treatment protocol and safety

There is no indication that the type of dose increase (conventional or rush) can influence the efficacy of VIT. The challenge, however, with faster protocols is to preserve or improve safety, since a greater risk of SRs has been demonstrated during the incremental phase of VIT (6).

A review of the published literature on safety of rush and ultrarush VIT studies from 1978 to 2001, gave controversial results (7). The frequency of SRs ranged from 0% to 67.3%, with an average of 17.8% (7). These discrepancies may be due to several factors: the use of different classification systems for the severity of adverse reactions, different study populations, the use of different extracts and preparations (aqueous vs. depot), the presence or absence of co-morbidities in some populations, and pre-medication with antihistamine in some patients. Moreover, rare comparative studies are available. An ultrarush VIT protocol over 3.5 hours resulted in fewer SRs compared to 6-hour and 4-day protocols, which involved higher cumulative daily doses (8). What does emerge from these studies is that, as for the conventional protocol, rush regimens have caused more SRs in bee venom allergic patients than in vespid allergic patients. Sturm et al. (7) reported their 8-year experience with a four-day rush protocol in predominantly high-risk patients. The number of SRs was higher in patients treated with bee venom extract (12%) compared with those receiving yellow jacket venom extract (2%), and was considerably lower than the average of 17.8% reported in the literature.

Since 2002, several other retrospective studies concluded that treatment with rush and ultrarush protocols are tolerated at least as well as or even better than treatment with slower protocols (9). Again, there seems to be a difference between honeybee or vespid venoms with respect to the tolerability of the different protocols. In a study on 1055 subjects treated with VIT, patients who underwent VIT with vespid venom, but not those treated with honeybee venom, had significantly fewer SRs when the dose was increased within 2 days (SRs in 15%) compared with 4 (20%) or 7 (30%) days (10). Using an ultrarush protocol of 3.5 hours, Birnbaum reported that 13% of 258 patients treated with honeybee or vespid venoms developed a SR (11), whereas Reimer reported that 26% of 57 honeybee allergic patients developed an SR (12). Also, in the recent study by Roumana and colleagues, using rush and ultrarush protocols, the great majority of SRs were caused by bee venom injections, at doses greater than 50 µg (3).

Side effects during VIT are probably more frequent in mastocytosis patients, especially in those with a yellow jacket venom allergy (13). At the moment there are very few data on rush and ultrarush protocols in these patients, in which a conventional built-up phase is in general favourite.

Rush and ultrarush protocols have typically been used instead of conventional VIT and not in patients who have experienced adverse reactions during conventional VIT. In case reports, rush and ultrarush protocols seem to be applicable and also tolerated in patients with a history of systemic reaction during conventional VIT; one patient tolerated ultrarush VIT with mixed vespid and wasp venom together with premedication reaching a total dose of 400 µg of venoms (14). Further studies are required to confirm this finding.

Conclusions

Many studies demonstrate that rush protocols are as safe as slower ones and confirm that treatment with faster protocols (ultrarush) are tolerated at least as well as or even better than treatments with slower rush protocols. However, these studies are mainly retrospective. Controlled prospective studies must be carried out to evaluate if the type of dose increase can influence the safety of VIT. There is evidence from these studies that there may be a difference between honeybee or vespid venoms with respect to the tolerability of the different protocols. At present, rush and ultrarush protocols should be used only by specialists with a great experience in

managing VIT, under emergency conditions, especially in bee venom allergic patients.

Disclosure
The author states no conflicts of interest.

References

1. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;**60**:1459-1470.
2. Diwakar L, Noorani S, Huissoon AP, Frew AJ, Krishna MT. Practice of venom immunotherapy in the United Kingdom: a national audit and review of literature. Clin Exp Allergy 2008;**38**:1651-1658.
3. Roumana A, Pitsios C, Vartholomaios S, Kompoti E, Kontou-Fili K. The safety of initiating Hymenoptera immunotherapy at 1 microg of venom extract. J Allergy Clin Immunol 2009;**124**:379-381.
4. Baenkler HW, Meusser-Storm S, Eger G. Continuous immunotherapy for hymenoptera venom allergy using six month intervals. Allergol Immunopathol (Madr). 2005;**33**:7-14.
5. Goldberg A, Confino-Cohen R. Effectiveness of maintenance bee venom immunotherapy administered at 6-month intervals. Ann Allergy Asthma Immunol 2007;**99**:352-357.
6. Mosbech H, Muller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. European Academy of Allergology and Clinical Immunology. Allergy 2000;**55**:1005-1010.
7. Sturm G, Kränke B, Rudolph C, Aberer W. Rush Hymenoptera venom immunotherapy: a safe and practical protocol for high-risk patients. J Allergy Clin Immunol 2002;**110**:928-933
8. Birnbaum J, Charpin D, Vervloet D. Rapid Hymenoptera venom immunotherapy: comparative safety of three protocols. Clin Exp Allergy 1993;**23**:226-230.
9. Rueff F, Przybilla B: Venom immunotherapy: adverse reactions and treatment failure. Curr Opin Allergy Clin Immunol 2004;**4**:307-311.
10. Brehler R, Wolf H, Kütting B, Schnitker J, Luger T. Safety of a two-day ultrarush insect venom immunotherapy protocol in comparison with protocols of longer duration and involving a larger number of injections. J Allergy Clin Immunol 2000;**105**:1231-1235.
11. Reimers A, Hari Y, Müller U. Reduction of side-effects from ultrarush immunotherapy with honeybee venom by pretreatment with fexofenadine: a double-blind, placebo-controlled trial. Allergy 2000;**55**:484-488.
12. Birnbaum J, Ramadour M, Magnan A, Vervloet D. Hymenoptera ultra-rush venom immunotherapy (210 min): a safety study and risk factors. Clin Exp Allergy 2003;**33**:58-64.
13. Niedoszytko M, de Monchy J, van Doormaal JJ, Jassem E, Oude Elberink JN. Mastocytosis and insect venom allergy: diagnosis, safety and efficacy of venom immunotherapy. Allergy 2009;**64**:1237-1245.
14. Oren E, Chegini S, Hamilos DL. Ultrarush venom desensitization after systemic reactions during conventional venom immunotherapy. Ann Allergy Asthma Immunol 2006;**97**:606-610.

PART FIVE

Poster session

DISPLAY OF WASP VENOM ALLERGENS ON THE CELL SURFACE OF YEAST

Irina Borodina[1], with Bettina Jensen[2], Ib Søndergaard[1], Jørgen Nedergaard Larsen[3], and Lars K. Poulsen[2]

[1]Center for Microbial Biotechnology, Institute of Systems Biology, Technical University of Denmark, Copenhagen, Denmark [2]Laboratory for Medical Allergology, Copenhagen, Denmark [3]Research Department, ALK-Abelló, Hørsholm, Denmark

Yeast surface display is a technique whereby proteins of interest are expressed in yeast as fusions with cell surface proteins and remain attached to the cell wall after expression (1, 2). The aim of this study was to investigate whether allergens preserve their native allergenic properties when expressed as fusion proteins on the surface of baker's yeast (*Saccharomyces cerevisiae*). We expressed the major allergens: phospholipase A1 (PLA1), hyaluronidase (HYA), and antigen 5 (Ves v 5), from the venom of the common wasp, *Vespula vulgaris*, as fusions with a-agglutinin complex protein AGA2. In order to express PLA1 (Ves v 1) and HYA (Ves v 2a), the surface display expression vector was modified to include an antibiotic resistance cassette that enabled a constant selective pressure to be applied to the cell population (Fig. 1). Cells were then examined for allergen expression by screening for enzymatic activity, in the case of PLA1 and HYA, binding of human IgE, and for the ability to release histamine from basophils.

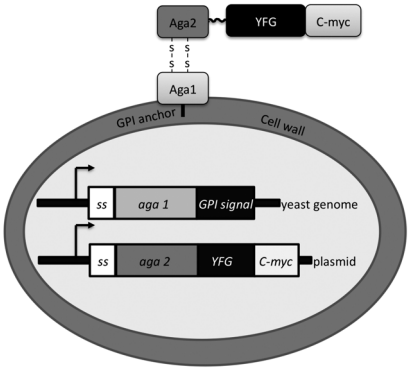


Fig. 1. The principle of surface display using a-agglutinin system.
The allergen of interest (YFG - your favourite gene) is cloned in-frame with AGA2 and introduced into a yeast strain that can over-express AGA1. The resulting proteins Aga1 and Aga2-allergen fusion are connected by disulfide bonds during the secretion process and exported together on the surface of the cell where Aga1 remains attached to the cell wall by the glycosylphosphatidylinositol (GPI) anchor.

All of the allergens were expressed on the surface of yeast cells. PLA1 and HYA both retained their enzymatic activity and the expression of PLA1 severely inhibited cell growth. Expression of HYA and antigen 5 was confirmed by fluorescent cytometry (FACS) of cells labelled with FITC-conjugated anti-C-myc antibody; the level of PLA1 expression, however, was too low to be detected by FACS. Yeast cells expressing HYA, bound IgE antibodies from the serum of venom-hypersensitive and control patients, whereas antigen 5 expressing cells bound IgE antibodies from the sera of venom-hypersensitive patients only. Double staining with an anti-C-myc antibody (FITC) and human IgEs, showed that expression of the full-length allergen correlated with IgE binding.

We used FACS, histamine release tests, and immunoblotting to investigate possible variations in the binding of antigen 5 expressed on the surface of yeast cells with sera from different venom-hypersensitive patients. The serum from one of five venom-hypersensitive patients did not react with Ves v 5 and the other four bound to different extents. Variations in antigen 5 binding to the different sera were confirmed by immunoblotting using recombinant antigen 5.

All the allergen-expressing cells caused histamine release from peripheral whole blood basophils charged with IgEs from hypersensitive serum (Fig. 2). PLA1-expressing cells caused the greatest release of histamine and also caused some response from basophils charged with IgEs from control sera. HYA-expressing cells were the least effective in causing histamine release.

In conclusion, the allergens expressed on the surface of yeast retained their IgE binding capabilities, enzymatic activity, and caused histamine release from basophils. Moreover, allergen-presenting yeast cells can be produced in large amounts at a low cost. These properties make yeast surface display a useful tool for allergen discovery in cDNA libraries (3) and a promising system for allergen presentation for sublingual specific immunotherapy.

Disclosure

The authors state no conflicts of interest.

References

1. Kondo A, Ueda M. Yeast cell-surface display - applications of molecular display. Appl Microbiol Biotechnol 2004;**64**:28-40.
2. Chao G., Lau W.L., Hackel B.J., Sazinsky S.L., Lippow S.M., and Wittrup K.D. Isolating and engineering human antibodies using yeast surface display. Nature Protocols 2006;**1**:755-768.
3. Wadle A, Mischo A, Imig J, Wüllner B, Hensel D, Wätzig K et al. Serological identification of breast cancer-related antigens from a Saccharomyces cerevisiae surface display library. Int J Cancer 2005;**117**:104-113.

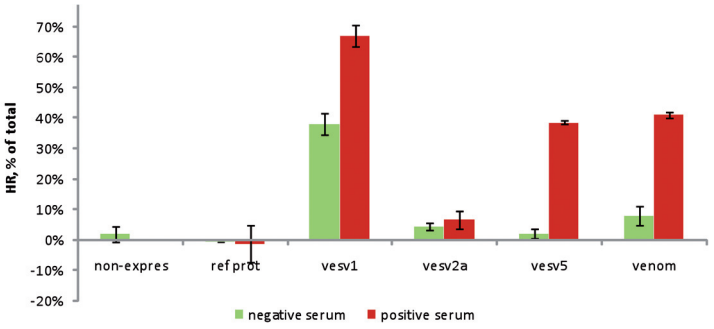


Fig. 2. Histamine release by allergen-expressing yeast cells.
Allergen-expressing cells (Ves v 1/PLA1; Ves v 2a/HYA; Ves v 5/antigen 5) had differing abilities to release histamine from peripheral whole blood basophils charged with hypersensitive and control serum IgEs (www.reflab.dk). Whole *Vespula vulgaris* venom was used as a positive control. Negative controls were either non expressing yeast cells ("non-expres") or yeast cells expressing a non-specific protein ("ref prot").

SUBLINGUAL IMMUNOTHERAPY OF HYMENOPTERA VENOM ALLERGY: A FOLLOW-UP OF EIGHT PATIENTS

Alessandro Buonomo, with Domenico Schiavino, Tiziana De Pasquale, Carla Lombardo, Valentina Pecora, Vito Sabato, Anegla Rizzi, Arianna Aruanno, Lucilla Pascolini, Eleonora Nucera, Giampiero Patriarca

Department of Allergology, Catholic University, Rome, Italy

Subcutaneous immunotherapy is presently the only effective treatment of Hymenoptera venom allergy. In the treatment of respiratory allergy, sublingual immunotherapy (SLIT) has been accepted worldwide as an effective and a safe alternative to subcutaneous allergen administration. The aim of this pilot study was to assess the tolerability, safety and efficacy of SLIT in patients with a systemic allergic reaction to Hymenoptera venom.

Eight patients (7 male and 1 female, age 37.6 ± 21.5 years [mean ± SD]) with Hymenoptera venom allergy (7 allergic to wasp venom and 1 to bee venom) were treated with an ultrarush SLIT using Hymenoptera venom. During 3 hours, patients received increasing doses of *Vespula* or bee venom (Aquagen®, ALK) up to a final dose of 30 drops (1 mL) of extract, containing a total of 100,000 SQ-U. Then patients received a maintenance dose of 10 drops of pure venom extract three times a week, corresponding to a total weekly dose of 100,000 SQ-U. Specific IgE (sIgE) and IgG4 were measured at 6, 12, 24 and 36 months.

All 8 patients were field stung during the maintenance phase, a mean of 12.7 ± 11.2 months after starting SLIT, and all had a lower symptom score compared with pretreatment stings. Five patients experienced only mild local reactions, 1 had throat constriction that did not need any treatment, 1 had eyelid angioedema that required treatment with bethametasone p.o. and 1 had no symptoms. All patients had experienced a Grade II or III (according to Müller) reaction before starting treatment. However, despite these encouraging clinical findings, we ob-

Dilution	Dose
1:10,000	1 drop
1:1,000	1 drop
1:100	1 drop
1:10	1 drop
Pure	1 drop
Pure	2 drops
Pure	4 drops
Pure	6 drops
Pure	7 drops
Pure	10 drops

Fig. 1. SIT ultrarush protocol adapted from (1).

served no significant modification of sIgE and IgG4 (Fig. 2).

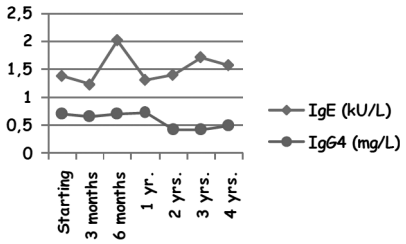


Fig. 2. Mean levels of specific IgE and IgG4 were not significantly modified during SLIT.

In conclusion, these results, despite being from a small number of subjects, indicate that SLIT may be a safe and effective approach to treat Hymenoptera venom allergy. However, several points should be taken into account in future studies: larger groups of patients are needed in order to assess the efficacy of SLIT; the optimal maintenance dose must be clearly defined, and should take into consideration cost as well as the risk/benefit ratio for the patient; and because of the variability between field stings, patient reaction should be tested using a controlled sting challenge. Future studies using an ultrarush SLIT protocol should also include a control group of patients treated with conventional SLIT to compare the effectiveness of both protocols in SLIT.

Disclosure

The authors state no conflicts of interest.

References

1. Patriarca G, Nucera E, Roncallo C, Aruanno A, Lombardo C, Decinti M, et al. Sublingual desensitization in patients with wasp venom allergy: preliminary results. Int J Immunopathol Pharmacol 2008;**21**:669-677.

REVIEW OF PRACTICES IN HYMENOPTERA VENOM IMMUNOTHERAPY - POLAND vs. the UNITED KINGDOM

Ewa Cichocka-Jarosz[1], with Piotr Brzyski[2], Beata Tobiasz-Adamczyk[2], Grzegorz Lis[1], Jacek J. Pietrzyk[1]

[1]Department of Pediatrics and [2]Department of Medical Sociology,
Polish-American Children's Hospital, Jagiellonian University Medical College, Cracow, Poland

Successful treatment of insect venom allergy requires the use of diagnostic tools that are capable of identifying the culprit insect and a therapeutic approach that is well adapted to the severity of the allergy. The aim of this study was to compare the approaches to diagnosis and treatment of Hymenoptera venom allergy in Poland and the United Kingdom. A questionnaire, adapted from the published study by Diwakar and collegues (1), containing 20 multiple choice and four short-answer questions on current procedures was sent to centres performing venom immunotherapy (VIT) in the UK and Poland. Centres in the UK were selected from the listing on the British Society for Allergy and Clinical Immunology (BSACI) website. The list of centres in Poland, which were academic (14), hospital (8) and non-public (4), respectively, was available on Polish Allergology Society website.

In Poland, 69% of centres used both specific IgE (sIgE) and skin prick tests to diagnose insect venom allergy, whereas in the UK, 55% of centres used sIgE tests alone (Fig. 1). Intradermal testing was carried out by 88% of centres in Poland compared with only 50% in the UK (2-4). In Poland, the insect venom extracts Venomenhal® (HALAllergy) and Pharmalgen® (ALK) were used for diagnostic purposes by 60% and 20% of centres respectively, and 16% of centres used both. The HALAllergy extract was used for treatment in 42% of Polish centres and the ALK product in 19%. In the UK, the ALK Abelló product was used by 95% for diagnosis and in 97% for treatment.

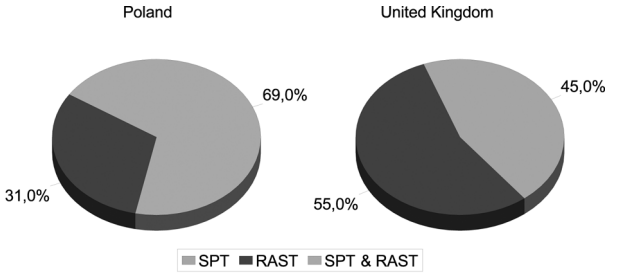


Fig. 1. sIgE testing was used as the only means of diagnostic testing by 55% of UK centres. Both sIgE and skin prick tests were used in first line of diagnostic procedures in 69% of centres in Poland.

In patients where the insect that caused the reaction cannot be identified and who have sIgE to both bee and wasp venom, treatment is started with the venom having the higher IgE level in the majority of UK centres, and in one third of centres in Poland. In the case of systemic reactions, baseline tryptase levels were measured in 39% of centres in Poland versus 53% in the UK (5). For initial treatment, a conventional protocol was preferred by 92% of UK centres; whereas in Poland 77% of centres preferred accelerated protocols (Fig. 2). Premedication with antihistamines was given to all the patients in 70% of Polish centres, while in the UK 60% of centres gave antihistamines although these were mainly confined to local troublesome reactions (6). The maximum interval most frequently used during the maintenance phase was 6 weeks in Poland and eight weeks in the UK and the target maintenance dose most frequently used in the two countries was 100 µg/ml. The majority of treatment centres in Poland considered five years to be the optimal treatment duration, while in the UK, three years was preferred (2-4). After completion of VIT, sting challenge testing, measurement of sIgE, and measurement of specific IgG4 (sIgG4) were performed by 46%, 46 % and 8% of Polish centres, respectively. In the UK, 11% of centres performed sting challenge testing, 47% measured sIgE, and none measured sIgG4.

To conclude, some differences in adherence to international guidelines for the diagnosis and treatment of Hymenoptera venom allergy exist between Poland and UK. Some of these might be a result of different organisation of health service in the country. A more thorough analysis is in preparation.

Disclosure

The authors state no conflicts of interest.

References

1. Diwikar L, Noorani S, Huissoon AP, Frew AJ, Krishna MT. Practice of venom immunotherapy in the United Kingdom: a national audit and review of literature. Clin Exp Allergy 2008;**38**:1651-8.
2. Moffitt JE, Golden DB, Reisman RE, Lee R, Nicklas R, Freeman T, et al. Stinging insect hypersensitivity: a practice parameter update. J Allergy Clin Immunol 2004;**114**:869-86.
3. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy 2005;**60**:1339-49.
4. Bonifazi F, Jutel M, Bilo BM, Birnbaum J, Muller U. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;**60**:1459-70.
5. Przybilla B., Rueff F. Hymenoptera venom allergy. J Dtsch Dermatol Ges 2009;epub available on-line.
6. Reimers A, Hari Y, Muller U. Reduction of side-effects from ultrarush immunotherapy with honeybee venom by pretreatment with fexofenadine: a double-blind, placebo-controlled trial. Allergy 2000;**55**:484-88.

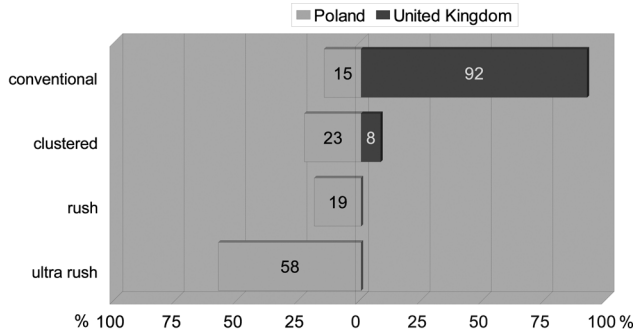


Fig. 2. A conventional protocol was used in 92% of UK centres, whereas 58% of Polish centres used ultrarush, and 19 % used rush. In Poland some centres practised more than one protocol.

KOUNIS SYNDROME IN A VENOM ALLERGIC PATIENT

Marta Piantanida, with Valerio Pravettoni, Laura Primavesi

Clinical Allergy and Immunology Unit, IRCCS Foundation Ospedale Maggiore Policlinico, Milan, Italy

Myocardial infarction and arrhythmia have been described following bee, wasp or hornet stings. During a systemic allergic reaction, mast cells in heart tissue release cytokines and vasoactive pro-inflammatory mediators that contribute to systemic anaphylaxis. Release of histamine induces vasodilatation and increases vascular permeability causing hypotension, however subsequent release of vasoconstrictor compounds can lead to coronary vasospasm, progressing to myocardial ischemia, heart failure and ventricular fibrillation (1). The occurrence of acute coronary syndromes accompanying allergic reactions is defined as Kounis syndrome or allergic angina/myocardial infarction (2, 3). Kounis syndrome is classified in two types: Type I occurs in patients with normal coronary vessels, while type II occurs in patients with concomitant atheromatic lesions. Both types are associated with coronary vasospasm. Only a few cases of Kounis syndrome have been described following Hymenoptera sting (4-6), however, there have been sporadic reports of myocardial ischemia, sometimes without other signs of anaphylaxis, after a Hymenoptera sting (7-10).

Here we describe a case of acute myocardial ischemia as a symptom of Hymenoptera venom anaphylaxis. A 41 year-old man fainted suddenly and was taken to an Emergency Care Unit where an electrocardiogram revealed acute anterior-lateral myocardial infarction, worsened by repeated episodes of ventricular fibrillation. Blood laboratory findings showed increased cardiac troponin-I (12.63 ng/mL; normal level < 1 ng/mL), creatin kinase isozymes MB to 58.40 ng/mL; normally <6 ng/mL) and aspartate transaminase to 219 U/L; normally <45 U/L). When conscious, the patient remembered receiving a Hymenoptera sting just before fainting. The patient had normal cardiac function and coronary arteries and was discharged with diagnosis of "acute myocardial infarction by coronary arterial vasospasm as result of severe anaphylaxis" and referred to the Allergology Unit of the hospital. The patient had a history of a sting by an unidentified Hymenoptera ten years previously that was followed by intense dizziness. The patient's specific IgE were positive for *Vespula spp.* (24.50 kU/L), *Polistes* (1.15 kU/L), and *Apis mellifera* (0.22 kU/L), and negative for *Vespa crabro* venom. The patient was started on specific immunotherapy for *Vespula spp.* venom. Kounis syndrome is rare, this is the only case of cardiac ischemia as unique symptom of anaphylaxis we have encountered in a population of around 4,000 patients with Hymenoptera venom allergy.

This case suggests that when cardiac ischemia of unknown aetiology occurs, hymenoptera sting should be considered as a possible cause and an electrocardiogram is

recommended after Hymenoptera allergic reaction, as myocardial infarction may be asymptomatic (4).

Disclosure

The authors state no conflicts of interest.

References

1. Perskvist N, Söderberg C, van Hage M, Edston E. Pathogenic role of cardiac mast cell activation/degranulation,TNF-alpha, and cell death in acute drug-related fatalities. Vasc Health Risk Manag 2007;**3**:1053-62.
2. Kounis NG, Zavras GM. Histamine-induced coronary artery spasm: the concept of allergic angina. Br J Clin Pract 1991;**45**:121-128.
3. Sinkiewicz W, Sobański P, Bartuzi Z. Allergic myocardial infarction. Cardiol J 2008;**15**:220-225.
4. Lombardi A, Vandelli R, Cerè E, Di Pasquale G. Silent acute myocardial infarction following a wasp sting. Ital Heart J 2003;**4**:638-641.
5. Hamilos MI, Kochiadakis GE, Skalidis EI, Igoumenidis NE, Zaharaki A, Vardas PE. Acute myocardial infarction in a patient with normal coronary arteries after an allergic reaction. Hellenic J Cardiol 2005;**46**:79-82.
6. Quercia O, Emiliani F, Foschi FG, Stefanini GF. Ventricular fibrillation after a hymenoptera sting. Int J Cardiol 2008;**127**:e5-7.
7. Calveri G, Bertelli Y, Caico SI, Ermolli NC, Torretta M, Lattanzio M, Salerno-Uriarte JA. Acute myocardial infarction after wasp sting. Ital Heart J Suppl 2002;**3**:555-557.
8. Bongo AS, Fornaro G, Sansa M, Macciò S, Rognoni A. Acute myocardial infarction after wasp sting without anaphylactic reaction. Ital Heart J Suppl 2005;**6**:178-182.
9. Korantzopoulos P, Kountouris E, Voukelatou M, Charaktsis I, Dimitroula V, Siogas K. Acute myocardial infarction after a European hornet sting - a case report. Angiology 2006;**57**:383-386.
10. Cvetković-Matić D, Asanin M, Matić D, Ivanović B, Simić D, Kalezić N, Stojanov Acute myocardial infarction following a hornet sting. V Vojnosanit Pregl 2009;**66**:333-337.

XYLOCOPA VIOLACEA: A RARE CAUSE OF ANAPHYLAXIS

Marta Piantanida[1], with Marina Mauro[2], Laura Primavesi[1], Danilo Labardi[3], Igino Spadolini[3], Valerio Pravettoni[1]

[1]Unit of Clinical Allergy and Immunology, Foundation Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, IRCCS, Milan, Italy; [2]Allergy Unit, S. Anna Hospital, Como, Italy; [3]Anallergo Laboratories, Florence, Italy

Xylocopa violacea, commonly known as the carpenter bee, is a solitary bee that is widespread in southern Europe (1). *Xylocopa violacea* measures up to 25 mm in length, is usually not aggressive and rarely stings. The medical literature does not contain any reports of anaphylaxis caused by an allergic reaction to a *Xylocopa violacea* sting and there is no information available on the allergenic components of *Xylocopa violacea* venom. Here we present two cases of anaphylaxis caused by *Xylocopa violacea* stings and identify the IgE-reactive allergens in *Xylocopa violacea* venom by immunoblotting after protein gel electrophoresis (SDS-PAGE).

Two patients experienced systemic anaphylaxis after *Xylocopa violacea* stings. Both patients were also stung by yellow jackets before and after anaphylaxis, without any reaction. We performed tests for venom specific serum IgE antibodies, venom skin prick test and intradermal tests (2, 3) with yellow jacket (YJ), paper wasp (W), European hornet (EH), honeybee (HB) and *Polistes dominulus* venoms. Five patients with negative allergic history were used as negative controls. *Xylocopa violacea* (Xy) extract was obtained from Anallergo (Florence, Italy). We performed SDS-PAGE and immunoblotting on all the above-mentioned venoms using a previously described methodology (4). The gel was blotted on a nitrocellulose membrane, before being cut into strips and matched with the sera of the two *Xylocopa violacea* allergic patients. After incubation with anti-IgE antibodies, the allergenic bands were detected by chemiluminescence.

In the two allergic patients, skin tests and serum specific IgE detection were positive only for yellow jacket extract. In one patient, there was light binding of IgE to the HB immunoblot, whereas in both allergic patients the *Xylocopa violacea* extract had IgE reactive bands at 25.2 kDa, 19.1 kDa and 6.5 kDa (Fig. 1). No binding was seen in control sera. The band at 19.1 kDa seemed to be specific for *Xylocopa violacea* venom. A 25.2 kDa allergen was also recognised in EH and YJ venoms, thus indicating a potential for possible cross-reactivity between *Xylocopa* and *Vespid* venoms. Any potential cross-reactivity, however, did not result in anaphylaxis as both patients were also stung by yellow jacket without experiencing a systemic reaction. IgE inhibition was not performed as there was no venom remaining. The 6.5 kDa band in HB and Xy venom could correspond to mellitin, surprisingly, serum specific IgE from allergic patients bound only to the 6.5 kDa band from *Xylocopa violacea*

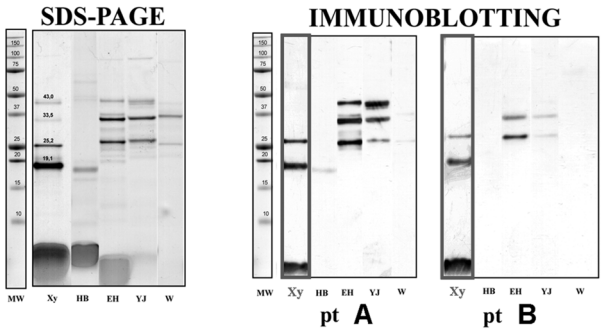


Fig. 1. SDS-PAGE gel and immunoblots of serum from *Xylocopa violacea* allergic patients (patients A and B) with venom from *Xylocopa violacea* (Xy), honeybee (HB), European hornet (EH), yellow jacket (YJ), and paper wasp (W) venoms.

extract, suggesting that if this is mellitin a difference in the allergen must exist between different members of the *Apidae* family.
In both cases of *Xylocopa violacea* venom anaphylaxis the responsible allergens seem to be the proteins at 19.1 kDa and probably at 6.5 kDa. Because of the rarity of this insect we encountered problems collecting venom for further studies. Planned further studies will include a cross-inhibition study and sequencing of the allergenic proteins.

Disclosure
The authors state no conflicts of interest.

References

1. Remko L, Cooper SJB, Schwarz MP. Molecular Phylogeny of the Large Carpenter Bees, Genus Xylocopa (Hymenoptera: Apidae), Based on Mitochondrial DNA Sequences. Mol Phylogen Evol 2000;**17**:407-418.
2. Bonifazi F, Jutel M, Bilò BM, Birnbaum J, Muller U, EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;**60**:1459-1470
3. Dreborg S. Skin tests used in type 1 allergy testing. Position paper. Allergy 1989;**44**:22-30.
4. Bonadonna P, Caruso B, Labardi D, Dama A, Senna G, Passalacqua G. Treatment with American Polistes venom was ineffective in an Italian patient allergic to European Polistes. Allergy 2007;**62**:966-7

HYMENOPTERA VENOM IMMUNOTHERAPY WITH MODIFIED RUSH PROTOCOL: EFFICACY AND SAFETY IN 114 CASES

Antonio Pio[1], with Francesco Pezzuto[1], Roberta Pio[2], Mario Lo Schiavo[1], M. Carmela Montera[1], Corrado Astarita[2]

[1]Allergy Clinic P.O. Mercato S. Severino, Salerno, Italy and [2]Second University of Napoli, Naples, Italy

The effectiveness of VIT in preventing anaphylaxis in Hymenoptera venom allergy has been documented by many prospective controlled studies, however, the safety and tolerability of VIT can differ between bee and wasp venom allergic patients. To investigate if a modified rush protocol could reduce the incidence of adverse reactions during VIT, we changed the rush protocol suggested by P.P. EAACI in 1993 (1,2). The modified protocol was tested on 114 patients (29 females and 85 males; mean age 44.3 years) and was adapted for adults and children (Table 1). Fifty patients (43.8%) had a history of Grade

Table 1. Modified rush protocol

Day	Adults minutes	Injected dose (µg)	Children minutes	Injected dose (µg)
1	0 45 90 135	0.01 0.1 1 2	0 45 90 135	0.01 0.1 1 2
2	0 45 90 135	3 6 12 20	0 45 90 135	3 5 10 15
3	0 45	40 60	0 45	30 50
4	-	100	-	80
11	-	100	-	85
25	-	-	-	90
46	-	-	-	95
74	-	-	-	100

Table 2. Numbers of patients experiencing adverse reactions during VIT with the modified rush protocol: 48. LR (local reaction): 38, LLR (large local reaction): 8, SR (systemic reaction; Müller severity scale): 2.

Reaction	*A. mellifera*	*V. crabro*	*Polistes spp.*	*Vespula spp.*
LR	3	15	3	17
LLR	1	4	0	3
SR I	0	1	0	0
SR II	0	0	0	0
SR III	1	0	0	0
SR IV	0	0	0	0

III systemic reactions and 64 patients (56.1%) had a history of Grade IV reactions. Skin prick tests and serum specific IgE antibodies indicated Hymenoptera venom sensitivity. Patients were treated with the same venom that had previously caused a systemic reaction; 19 patients were treated with *Apis mellifera* venom, 15 with *Polistes spp.* venom, 32 with *Vespa crabro* venom and 48 with *Vespula spp.* venom. Patients were continually monitored for adverse reactions throughout treatment.

A total of 7068 injections (1254 during induction phase and 5814 during maintenance phase) were performed during the study. Local reactions occurred in 38 patients (33.3%) and large local reactions in 8 patients (7.1%). Local reactions were induced by allergen doses between 20 and 60 µg. Two patients (1.6%) experienced systemic reactions (Grade I and III) during treatment. Thirty-nine patients (34.2%) who were stung during the treatment period suffered only local reactions (Table 2).

In conclusion, the modified rush protocol showed similar efficacy in preventing an anaphylactic reaction to Hymenoptera venom as those described in other studies, but with a lower dose during induction phase. Systemic adverse reactions occurred in 1.6% of the patients during dose-increase phase and they were absent during five years of maintenance phase. In some other studies using rush protocol between 5.9 and 67% of the patients had systemic reaction during induction phase (3-10).

Disclosure

The authors state no conflicts of interest.

References

1. Müller U, Mosbech H. Position Paper: Immunotherapy with Hymenoptera venoms. EAACI. Allergy 1993;**48**:36-46.
2. Müller U. Insect sting allergy: clinical picture, diagnosis and treatment. Stuttgart: Gustav Fischer Verlag, 1990.
3. Gorska L, Celmimska M, Kuziemski K, Skrzypski M, Niedoszytko M. Damps-Konstanska I et al. Analysis of safety, risk factors and pre-treatment methods during rush Hymenoptera venom immunotherapy. Int Arch Allergy Immunol 2008;**147**:241-245.
4. Sturm G, Kranke B, Rudolph C, Aberer W. Rush Hymenoptera venom immunotherapy: a safe and practical protocol for high-risk patients. J Allergy Clin Immunol 2002;**110**:928-933.
5. Brehler R, Wolf H, Kutting B, Schnitker J, Luger T. Safety of a two-day ultrarush insect venom immunotherapy protocol in comparison with protocols of longer duration and involving a larger number of injections. J Allergy Clin Immunol 2000;**105**:231-1235.
6. Mosbech H, Müller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. Allergy 2000;**55**:1005-1010.
7. Laurent J, Smiejan JM, Bloch-Morot E, Herman D. Safety of Hymenoptera venom rush immunotherapy. Allergy 1997;**52**:94-96.
8. Birnbaum J, Charpin D, Vervolet D. Rapid

Hymenoptera venom immunotherapy: comparative safety of three protocols. Clin Exp Allergy 1993;**23**:226-230.
9. Berchtold E, Maibach R, Muller U. Reduction of side effects from rush-immunotherapy with honney bee venom by pre-treatment with terfenadine. Clin Exp Allergy 1992;**22**:59-65.
10. Przybilla B, Ring J, Griesshammer B, Braun-Falco O. Schnellhyposensibilisierung mit Hymenopterengiften. Dtsch Med Wochenschr 1987;**112**:416-424.

CONCLUSION
Present and future of venom allergy: conclusions

Floriano Bonifazi and M. Beatrice Bilò

Allergy Unit, Department of Internal Medicine, Allergy, Immunology and Respiratory Diseases, University Hospital, Ancona, Italy

Allergic sting reactions are responsible for significant morbidity and deterioration in health-related quality of life. The latest epidemiological data confirm the importance of insect-sting allergy as one of the causes of anaphylaxis, which can be fatal (1). The combination of several concomitant factors, which include environmental, genetics, and individual risk factors are responsible for the occurrence of a systemic reaction (SR) in individual patients (2). Several well-known and recently discovered risk factors may be associated with the severity of a SR to a resting. The results of a multicentre prospective study performed by the EAACI Interest Group on Insect Venom Allergy examining the risk factors for severe SRs to insect sting in a large patient population has been recently published (3). Several risk factors were associated to severe SRs in untreated patients: an increase in baseline tryptase concentration, vespid stings, higher age, concomitant use of an angiotensin converting enzyme inhibitor, and a precedent less severe SR to a sting (3). Recently, Bonadonna and colleagues published the finding that 65% of patients with an elevated serum basal tryptase level (>11.4 ng/mL) and a history of sting SR have an underlying clonal mast cell disorder that is either systemic mastocytosis or monoclonal mast cell activation syndrome (4). Recent practice parameters on the diagnosis and treatment have been published by the European Academy of Allergology and Clinical Immunology (5) and by the American Academy of Allergy, Asthma and Immunology (6). However, the first comprehensive audit performed in the UK in order to evaluate adherence to international guidelines demonstrates a wide variation in practice of the diagnosis and treatment by allergy practitioners (7).

Pitfalls and future perspectives in venom allergy diagnosis

Some pitfalls for the unwary remain when diagnosing venom allergy: firstly, IgE-negative anaphylaxis and secondly, multiple venom sensitisation with the history of a single severe reaction to a sting. Table 1 shows some possible causes of IgE-negative venom anaphylaxis (8-12). In rare cases of IgE-negative anaphylaxis, additional *in vitro* methods, such as the basophil activation test, may be used to demonstrate immunological sensitisation (5). Further studies are needed to confirm the utility of a modified assay able to detect very low concentrations of venom specific IgE (13) and the use of a dialysed venom extract for skin testing (14). All relevant venoms according to insect geographic distribution should be tested (12), before concluding on alternative pathogenetic mechanisms.

Table 1. Possible causes of IgE-negative venom anaphylaxis

- Poor sensitivity of diagnostic tests (8)
- Loss of sensitisation over time (9)
- Involvement of a different pathogenetic mechanism (10)
- Mastocytosis/Urticaria pigmentosa (11)
- Lack of testing with causative insect (12)

The presence of multiple sensitisation with a single history of a SR represents the other side of the coin. In these cases, although the specific IgE inhibition test is helpful in distinguishing between cross-reactivity and double sensitisation, this is expensive, sometimes difficult to interpret and is therefore not suitable for routine purposes. According to some authors, the basophil activation test may be useful in multiple sensitive patients, provided that it is performed in validated laboratories (15). However, the diagnosis of venom allergy is currently performed with whole venom preparations, which contain other non-allergenic components in addition to allergens. A recent study by Müller suggested that the *in vitro* use of recombinant non-glycosylated major allergens could help in the interpretation of these difficult to diagnose patients (16). Recombinant venom allergens will certainly improve the diagnosis of venom allergy, also by clarifying cross-reactivity between venom allergens from different species, genera or even families of hymenoptera, provided that all major allergens specific to honeybee and Vespids become available in recombinant form. In the near future, the combination of molecular preparations and microtechnology for testing (microarray) will probably enable the physician to identify a patient's specific profile and produce a patient-tailored immunotherapy.

Pitfalls and future perspectives in venom immunotherapy

Venom immunotherapy (VIT) is highly effective treatment and is probably the most effective form of immunotherapy available to physicians. Although the mechanism involved is still not completely understood, the induction of a tolerant state in peripheral T-cells represents an essential step in allergen immunotherapy. Peripheral T-cell tolerance is characterised mainly by the generation of allergen-specific T-regulatory (Treg) cells and the production of their suppressive IL-10 cytokines and transforming growth factor-beta, which mainly suppress Th2 and Th1 cells, mast cells, basophils, eosinophils and regulate antibody isotypes in B cells (17). A rapid expansion of IL-10-producing Treg cells (so called Tr1 Treg) has been extensively demonstrated in bee venom immunotherapy.

However, some pitfalls remain in the management of allergic patients undergoing venom immunotherapy. Indeed, both efficacy and safety are not optimal, especially in immunotherapy with bee venom, where 10-20% of those treated still develop some, mostly mild, systemic allergic symptoms when re-stung during immunotherapy, and 20-30% develop systemic allergic side effects, especially during the dose step-up phase (18). Some current and future possibilities of enhancing VIT safety and efficacy are listed in Table 2. Pre-treatment with H1 antihistamines has been demonstrated to reduce the number and/or severity of large local reactions (LLRs) and mild SRs like urticaria and angioedema. Antihistamine pretreatment during the dose-increase phase may increase the long-term efficacy of VIT due to enhancement of Treg cell actions by activation of H2R-dependent events, although the current data are inconclusive and call for further investigation (19). Pre-treatment with anti-IgE monoclonal antibodies could permit more rapid and higher doses of allergen immunotherapy and may enhance its safety, even in patients suffering from systemic mastocytosis (20). At present, com-

Table 2. How to increase VIT safety and efficacy?

- Anti-IgE mAb pretreament (> safety) (20)
- "Depot" and purified aqueous extracts (> safety) (21,22)
- Modified allergens (?) (24)
- Intralymphatic route (?) (23)
- SLIT (?) (25)

pared to the therapy with an aqueous extract (both purified and non-purified), VIT with a depot extract is superior with respect to the occurrence of large LLRs and SRs (21). Purified extracts seem to be safer than the non-purified ones, especially concerning the occurrence of LLRs (22). The therapeutic potential of bee venom intralymphatic immunisation has been analysed in sensitised mice using an anaphylaxis model (23), and a chimeric protein containing six pieces of the three BV allergens PLA, hyaluronidase and melittin that was able to reduce IgE binding but still preserve T-cell stimulating activity has also been studied in a mouse model (24). Although sublingual venom immunotherapy (SLIT) seems to reduce the diameter of LLR to a bee sting challenge in 57% of active treated patients (25), caution should be exercised when considering SLIT as a therapeutic option for venom allergic patients.

Patients with mast cell and cardiovascular diseases, even if on pharmacotheraphy with beta-blockers, may be treated with VIT, albeit under strict supervision, including monitoring of blood pressure and an electrocardiogram during the dose-increase phase.

In Europe, the EAACI document "Standards for practical allergen-specific immunotherapy" (26) underlines that serious immunological diseases, cancer, chronic infections are absolute contraindications for immunotherapy in general as well as major cardiovascular diseases, except in the event of serious insect venom allergies. However, European guidelines need to be further discussed and possibly revised for severe allergic reactions due to Hymenoptera stings. This is particularly important in relation to patients with a high risk of sting exposure and with a history of a near fatal sting reaction where, in some cases, the advantages of venom immunotherapy might outweigh the potential risk.

Conclusions

For over 20 years now, new developments in the diagnosis of insect venom allergies and the administration of specific immunotherapy, have dramatically improved the prognosis of venom allergy. However, there is a considerable room for establishing more knowledge of the natural history and possible risk factors of venom allergy, as well as diagnosis, safety and efficacy of VIT.

Disclosure

The authors state no conflicts of interest.

References

1. Bilò BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. Curr Opin Allergy Clin Immunol 2008;**8**:330-337.
2. Bilò BM, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. Clin Exp Allergy 2009;**39**:1467-1476.
3. Ruëff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase-a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol 2009;**124**:1047-1054.
4. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal Fior D et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol 2009;**123**:680-686.
5. Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN; EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. Allergy 2005;**60**:1339-1349.
6. Moffitt JE, Golden DB, Reisman RE, Lee R, Nicklas R, Freeman T et al. Stinging insect hypersensitivity: a practice parameter update. J Allergy Clin Immunol 2004;**114**:869-886.
7. Diwakar L, Noorani S, Huissoon AP, Frew AJ, Krishna MT. Practice of venom immunotherapy in the United Kingdom: a national audit and review of literature. Clin Exp Allergy 2008;**38**:1651-1658.
8. Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Insect sting allergy with negative venom skin test responses. J Allergy Clin Immunol 2001;**107**:897-901.

9. Goldberg A, Confino-Cohen R.Timing of venom skin tests and IgE determinations after insect sting anaphylaxis.J Allergy Clin Immunol 1997;**100**:182-184.
10. Hermann K, Ring J. The renin-angiotensin system in patients with repeated anaphylactic reactions during hymenoptera venom hyposensitization and sting challenge. Int Arch Allergy Immunol 1997;**112**:251-256.
11. Fricker M, Helbling A, Schwartz L, Müller U. Hymenoptera sting anaphylaxis and urticaria pigmentosa: clinical findings and results of venom immunotherapy in ten patients. J Allergy Clin Immunol 1997;**100**:11-5.
12. Bilò MB, Brianzoni F, Cinti B, Napoli G, Bonifazi F. The dilemma of the negative skin test reactors with a history of venom anaphylaxis: will this always be the case? Eur Ann Allergy Clin Immunol 2005;**37**:341-342.
13. Guerti K, Bridts CH, Stevens WJ, Ebo DG. Wasp venom-specific IgE: towards a new decision threshold? J Investig Allergol Clin Immunol 2008;**18**:321-323.
14. Golden DB, Kelly D, Hamilton RG, Wang NY, Kagey-Sobotka A. Dialyzed venom skin tests for identifying yellow jacket-allergic patients not detectedusing standard venom.Ann Allergy Asthma Immunol 2009;**102**:47-50.
15. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: technical issues and critical factors. Allergy 2009;**64**:1319-1326.
16. Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and Vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy 2009;**64**:543-548.
17. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 2007;**119**:780-791.
18. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;**60**:1459-1470.
19. Müller UR, Jutel M, Reimers A, Zumkehr J, Huber C, Kriegel C et al. Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy. J Allergy Clin Immunol 2008;**122**:1001-1007.
20. Kontou-Fili K. High omalizumab dose controls recurrent reactions to venom immunotherapy in indolent systemic mastocytosis. Allergy 2008;**63**:376-378.
21. Ruëff F, Wolf H, Schnitker J, Ring J, Przybilla B. Specific immunotherapy inoneybee venom allergy: A comparative study using aqueous and aluminium hydroxide adsorbed preparations. Allergy 2004;**59**:589-595.
22. Bilò MB, Severino M, Cilia M, Pio A, Casino G, Ferrarini E et al. The VISYT trial: Venom Immunotherapy Safety and Tolerability with purified vs nonpurified extracts. Ann Allergy Asthma Immunol 2009;**103**:57-61.
23. Martínez-Gómez JM, Johansen P, Erdmann I, Senti G, Crameri R, Kündig TM. Intralymphatic Injections as a New Administration Route for Allergen-Specific Immunotherapy. Int Arch Allergy Immunol 2009;**150**:59-65.
24. Karamloo F, Schmid-Grendelmeier P, Kussebi F, Akdis M, Salagianni M, von Beust BR et al. Prevention of allergy by a recombinant multi-allergen vaccine with reduced IgE binding and preseved T cell epitopes. Eur J Immunol 2005;**35**:3268-3276.
25. Severino MG, Cortellini G, Bonadonna P, Francescato E, Panzini I, Macchia D et al. Sublingual immunotherapy for large local reactions caused by honey bee stings. J Allergy Clin Immunol 2008;**122**:44-88.
26. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E; EAACI, Immunotherapy Task Force. Standards for practical allergen-specific immunotherapy. Allergy 2006;**61** Suppl 82:1-20.

Supported by an unrestricted grant from

ISBN: 978-3-033-02268-3